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NUCLEAR OXIDATION IN THE FLAVONE SERIES

Part IX. Oxidation of Related Chalkones

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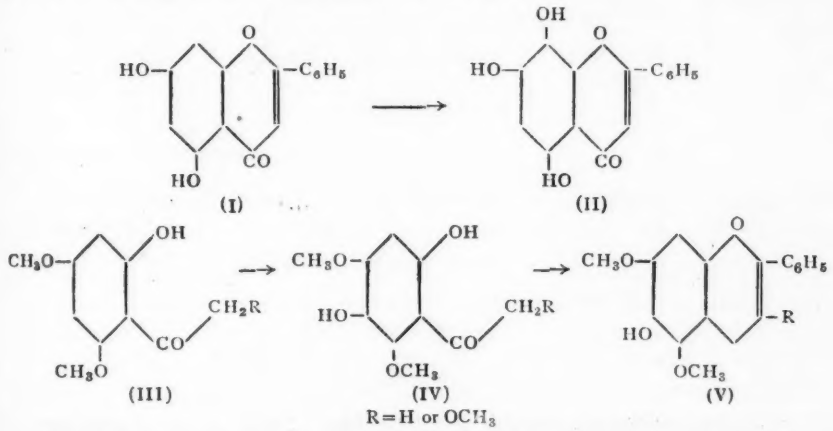
(From the Department of Chemistry, Andhra University, Waltair)

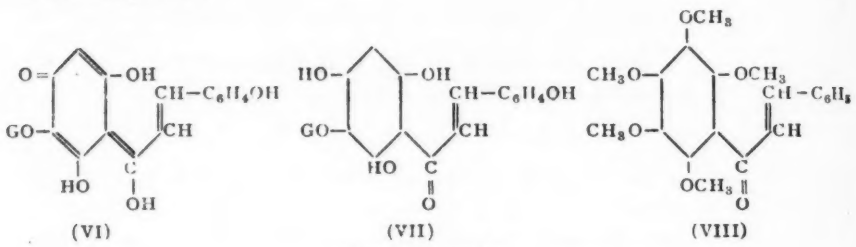
Received October 29, 1947

From the results recorded in the earlier papers of this series it is clear that the introduction of a hydroxyl group in the 8-position (II) of naturally occurring flavones by nuclear oxidation is quite facile and that this process is most possibly adopted in phytochemical synthesis. On the other hand the 6-position of the flavone structure does not appear to be available for oxidation and hence in the formation of flavones with a hydroxyl in the 6-position (baicalein and quercetagetin series) a precursor with the pyrone ring not yet closed would appear to be involved in the oxidation. As appropriate model experiments the oxidation of phloroacetophenone dimethyl ether and of its ω-methoxy derivative (III) could be mentioned.[1, 7] It was, however, considered desirable to study the behaviour in this oxidation of compounds like chalkones and dibenzoylmethanes which are closely related to the flavones and which can be eventually converted into flavones. The results of exploratory experiments using chalkones are described in this paper.



Besides their relationship to flavones, members of the chalkone group are otherwise also important because they occur in nature. Examples of

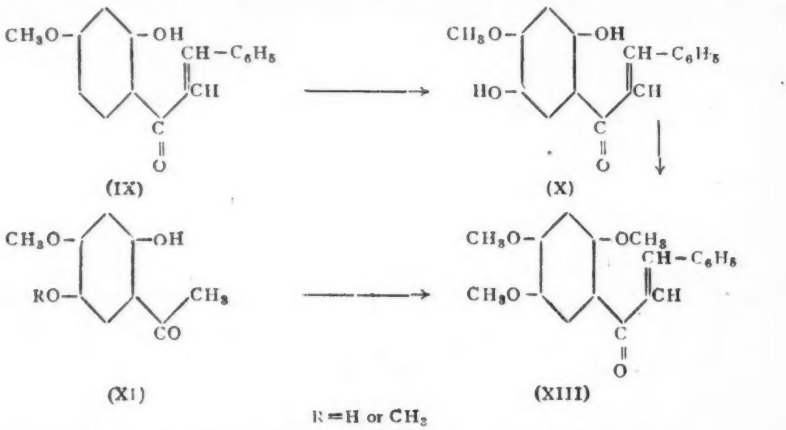
chalkones having additional hydroxyl groups in the phloroglucinol nucleus are found in carthamin (VI) and isocarthamin[2] (VII) which are glucosides and pedicellin (VIII)[3], pedicin and their allies which are fully or partly methylated compounds.



(VI) (VII) (VIII)

G = glucose residue

Experiments on the nuclear oxidation of chalkones are not only of interest in regard of biogenesis, but they could also be expected to be useful for the preparation of certain types of compounds which cannot be easily obtained from the appropriate ketone and aldehyde components by condensation. For example chalkone (X) is the direct result of the nuclear oxidation of chalkone (IX) whereas it cannot be conveniently made by the condensation of ketone (XI, R = H) with benzaldehyde. The presence of free hydroxyl groups and particularly *p*-dihydroxyl groups in the reacting components lead to complications and to failure in the condensation.



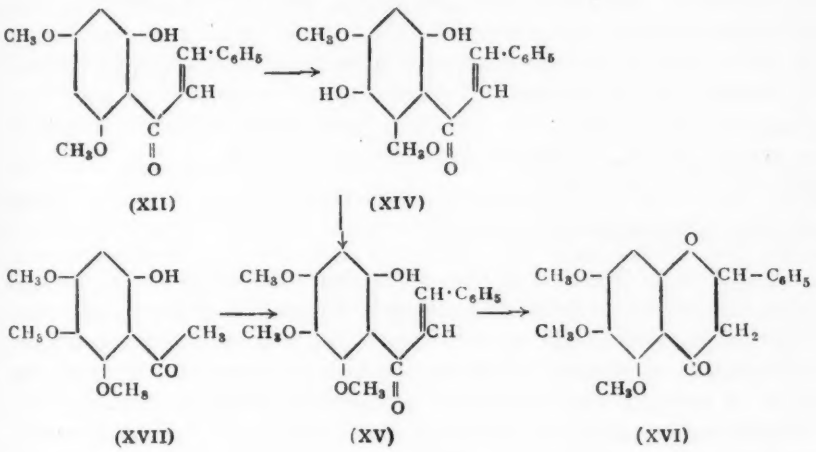
(IX) (X)

(XI) (XIII)

R = H or CH_3

2-Hydroxy-4-methoxy-chalkone (IX) and 2-hydroxy-4: 6-dimethoxy-chalkone (XII) have now been subjected to nuclear oxidation using alkaline persulphate. In order to get good yields of the products it is necessary to

use lower temperatures for the oxidation and gentler conditions for hydrolysing the sulphate formed as the intermediate stage. For confirming the constitution of the product (X) obtained in the first case, it has been fully methylated. The trimethyl ether is found to be identical with 2:4:5-trimethoxy chalkone (XIII) prepared from 2-hydroxy-4:5-dimethoxy-acetophenone (XI, R = CH_3) by condensation with benzaldehyde and subsequent methylation (*cf.* Bargellini *et al.*[4]). In the second case the dihydroxy-chalkone (XIV) has been partially methylated (XV) and then converted into the trimethoxy-flavanone which is found to be identical with 5:6:7-trimethoxy-flavanone (XVI) obtained independently from 2-hydroxy-4:5:6-trimethoxy-acetophenone (XVII) as indicated below:—



Experimental

2-*Hydroxy*-4-*methoxy-chalkone* (*IX*)

This compound was prepared earlier by Bargellini and Monti.[5] The following conditions employing excess of the aldehyde and ordinary room temperature give rise to good yields.

A solution of 2-hydroxy-4-methoxy-acetophenone (3·0 g.) and benzaldehyde (6 c.c.) in alcohol (20 c.c.) was cooled to 0° and potassium hydroxide (30·0 g. in 24 c.c. of water) was added in small quantities at a time with shaking. The flask was stoppered tight and kept for two days at the laboratory temperature. The contents were then diluted with water, extracted with ether to remove the unreacted aldehyde and then acidified. The chalkone which separated as an yellow solid was freed from benzoic acid by treatment

with ice-cold sodium bicarbonate solution. On recrystallisation from alcohol it was obtained as stout elongated hexagonal crystals melting at 105–6°, the m.p. recorded by Bargellini and Monti being 106–8°. Yield 3·0 g.

2: 5-*Dihydroxy*-4-*methoxy-chalkone* (X)

To a stirred solution of 2-hydroxy-4-methoxy-chalkone (IX) (1·0 g.) in a mixture of pyridine (10 c.c.) and aqueous sodium hydroxide (0·6 g. in 20 c.c.) were added dropwise potassium persulphate solution (1·5 g. in 50 c.c.) and aqueous sodium hydroxide (0·6 g. in 20 c.c.) during the course of 2 hours. After allowing the solution to stand for 24 hours, it was neutralised (congo-red) with dilute hydrochloric acid and extracted twice with ether. The clear brown aqueous solution was treated with sodium sulphite (2·0 g.) and concentrated hydrochloric acid (20 c.c.). On heating the solution at 100° for 15 minutes an orange-yellow solid separated out which was filtered and washed. When recrystallised from alcohol it separated as long stout rectangular plates melting at 168–70°. With alcoholic ferric chloride it gave a brown colour. Yield, 0·25 g. (Found: C, 71·6; H, 4·9; $C_{16}H_{14}O_4$ requires C, 71·1, H, 5·2%.)

2: 4: 5-*Trimethoxy-chalkone* (*XIII*)

A solution of the above 2: 5-dihydroxy-4-methoxy-chalkone (X) (0·2 g.) in dry acetone (20 c.c.) was treated with dimethyl sulphate (1 c.c.) and anhydrous potassium carbonate (5·0 g.). After refluxing for ten hours the potassium salts were filtered off and washed with warm acetone. On removing the solvent from the acetone solution the trimethoxy-chalkone was obtained as a bright yellow solid. It crystallised from alcohol as fine needles melting at 115–16°. Yield 0·15 g. Its solution in concentrated sulphuric acid was orange-red. A mixed melting point with the sample of 2: 4: 5-trimethoxy-chalkone obtained from 2-hydroxy-4: 5-dimethoxy-acetophenone (XI, $R = CH_3$) was undepressed. The condensation of this ketone (1·0 g.) with benzaldehyde (2 c.c.) was effected under conditions already described. 2-Hydroxy-4: 5-dimethoxy-chalkone (yield, 1·0 g.) crystallised from absolute alcohol as bright-yellow needles. Methylation with dimethyl sulphate and potassium carbonate yielded the trimethoxy chalkone melting at 115–16°.

2-*Hydroxy*-4: 6-*dimethoxy-chalkone* (*XII*)

This chalkone was prepared earlier by Kostanecki and Tambor.[6] By employing excess of aldehyde and following the procedure already mentioned in this paper better yields could be obtained. 2-Hydroxy-4: 6-dimethoxy-acetophenone (3·0 g.) when condensed with benzaldehyde (6 c.c.) yielded

the above chalkone (2·0 g.) as an orange coloured solid. It crystallised from alcohol as fine needles melting at 91–92°.

2: 5-*Dihydroxy*-4: 6-*dimethoxy-chalkone* (*XIV*)

To a stirred solution of 2-hydroxy-4: 6-dimethoxy-chalkone (XII) (1·0 g.) in a mixture of pyridine (10 c.c.) and aqueous sodium hydroxide (1·0 g. in 30 c.c.) was added dropwise potassium persulphate solution (1·5 g. in 40 c.c. of water) during the course of two hours. Further steps were as already described for a similar case. The crude dihydroxy compound was extracted with ether and recrystallised from aqueous alcohol when it was obtained as fine needles, melting at 156–58°. With alcoholic ferric chloride it gave a deep brown colour. Yield 0·2 g. (Found: C, 67·9; H, 5·5; $C_{17}H_{16}O_5$ requires C, 68·0; H, 5·3%.)

5: 6: 7-*Trimethoxy-flavanone* (*XVI*)

(i) A solution of the above 2: 5-dihydroxy-4: 6-dimethoxy-chalkone (1·0 g.) in dry acetone (20 c.c.) and dry benzene (40 c.c.) was treated with dimethyl sulphate (0·5 c.c.) and anhydrous potassium carbonate (5·0 g.). After refluxing for six hours the potassium salts were filtered and washed with warm benzene. The benzene filtrate was extracted with 5% aqueous sodium hydroxide four times. The combined alkaline extracts were run into ice-cold dilute hydrochloric acid, extracted with ether and the ether solution washed with 4% aqueous sodium carbonate. On distilling off the solvent 2-hydroxy-4: 5: 6-trimethoxy-chalkone (XV) was obtained as a bright yellow oil and could not be crystallised. With alcoholic ferric chloride it gave a reddish-brown colour. Yield 0·7 g. It was directly used for conversion into the flavanone. A solution of the chalkone (0·6 g.) in 50% aqueous alcohol (50 c.c.) was treated with concentrated sulphuric acid (1·5 c.c.) and the resulting solution boiled under reflux for 24 hours. On concentrating the solution the trimethoxy-flavanone separated out as a colourless crystalline solid. It was filtered and washed first with a weak solution of sodium hydroxide and then with water. On crystallisation from alcohol it was obtained as colourless needles melting at 154–55°. It gave no colour with alcoholic ferric chloride but a pink solution with magnesium and hydrochloric acid. Mixed melting point with the sample obtained by the method given below indicated no depression. (Found: C, 68·3; H, 5·7; $C_{18}H_{18}O_5$ requires C, 68·8; H, 5·7%.)

(ii) 2-Hydroxy-4: 5: 6-trimethoxy-acetophenone (XVII) was obtained by the method of Sastri and Seshadri.[7] It (1·0 g.) was condensed with benzaldehyde (3 c.c.) under the conditions employed in previous cases. The

product (XV) (0·6 g.) was again a bright yellow liquid and could not be crystallised. When converted into the flavanone it yielded 5: 6: 7-trimethoxy-flavanone (XVI) melting at 154–55° and identical with the sample described above in every respect.

Summary

It is shown that the nuclear oxidation of hydroxy-chalkones can be successfully effected thereby introducing a fresh hydroxyl in the 5-position which corresponds to the 6-position in flavanones and flavones. 2-Hydroxy-4-methoxy-chalkone and 2-hydroxy-4: 6-dimethoxy-chalkone have thus been oxidised to the 2: 5-dihydroxy compounds. In the first case the product is shown to be 2: 5-dihydroxy-4-methoxy-chalkone by complete methylation and identification of the derivative as 2: 4: 5-trimethoxy-chalkone. In the second case it is proved to be 2: 5-dihydroxy-4: 6-dimethoxy-chalkone by partial methylation and conversion into 5: 6: 7-trimethoxy-flavanone.

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NUCLEAR OXIDATION IN THE FLAVONE SERIES

Part X. Constitution of Gardenin

By K. J. Balakrishna and T. R. Seshadri

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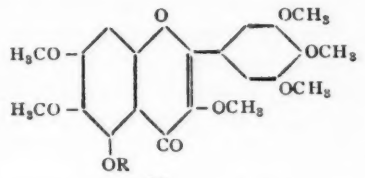
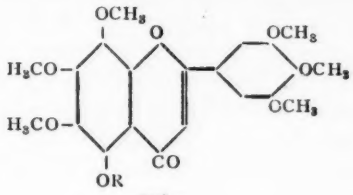
Received October 29, 1947

Gardenin is the yellow crystalline component of the Dikamali gum (gum of *Gardenia lucida*) which has been used in India as a drug. It was first isolated by Stenhouse[1] and the most recent work is by Bose[2] who made a detailed study of the compound and proposed a constitution for it. He showed that the substance belonged to the group of flavones, having one hydroxyl group and six methoxyl groups. The free hydroxyl was placed in the 5-position since gardenin was sparingly soluble in alkali, gave ferric chloride colour and a condensation product with stannic chloride and was resistant to further methylation. Degradation with alkali yielded trimethyl gallic acid as a readily identified product. The ketonic half had undergone further

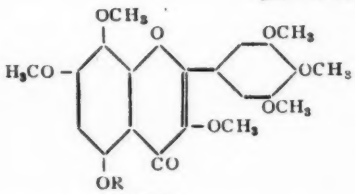


(I)

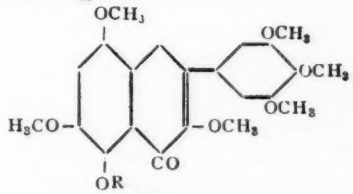


(II)

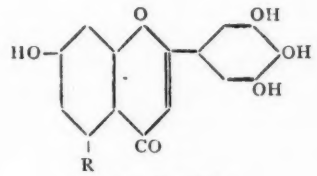
[In I to IV, R = H or CH_3]



(III)



(IV)



(V) R = H or OH

changes, oxidation and demethylation. The formation of the acid located three of the six methoxyl groups in the side-phenyl nucleus. There were four possible arrangements for the other three methoxyl groups in the benzopyrone part—3:6:7 (I), 6:7:8 (II), 3:7:8 (III), 3:6:8 (IV). Since crude nor-gardenin did not give Bargellini's test formulæ (I) and (II) were ruled out. Against formula (III) it was found that gardenin did not couple with diazotised *p*-nitraniline indicating the absence of a free nuclear position para or ortho to the phenolic hydroxyl. Further methyl gardenin was not identical with O-heptamethyl hibiscetin (III, R = CH_3). Hence by elimination of other possibilities structure (IV, R = H) was allotted by Bose to gardenin.

The above constitution for gardenin would make it a representative of an altogether unknown type of hydroxy-flavones. It was therefore felt necessary to adduce unequivocal evidence in support of this constitution and the application of the method of nuclear oxidation in the flavones has now offered an easy means of providing this support from the synthetic side.

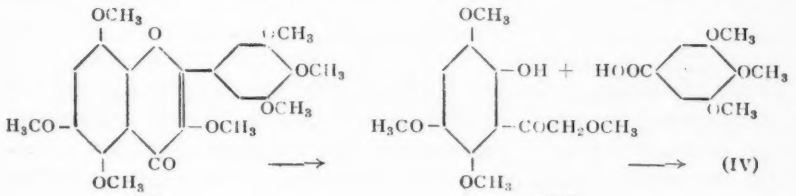
Before taking up the synthetic work a more detailed study of gardenin and of its derivatives was undertaken in order to make sure of the proposed structure. It is fairly readily obtained from the gum and an yield of 1 to 1·4% could be secured by adopting the procedure described in the experimental part. In our study of a large number of samples obtained at different times and from different places it was frequently found that several of them did not yield any gardenin, though apparently the gum samples looked genuine. But the absence of a marked yellow colour in the gum corresponded to lack of yields of gardenin. It seems to be therefore necessary to use deep yellow coloured gums in order to obtain good yeilds of the pigment.

The properties of our samples of gardenin agree with the description of previous workers. The methylation of gardenin is unusually difficult. Bose and Nath[2] obtained the methyl ether only as a pale yellow solid melting at 102° even after repeated methylation using dimethyl sulphate and aqueous sodium hydroxide. A sample of this that was sent to us, gave also a faint ferric chloride colour. Obviously the methylation was not quite complete by this process. However by using excess of dimethyl sulphate and potassium carbonate and boiling for 30 hours, a colourless product could now be obtained melting at 116–17°. Comparison of this pure product with (1) hibiscetin methyl ether[3] (III, R = CH_3), m.p. 194–6°, and (2) 6-hydroxy-myricetin methyl ether,[4] m.p. 150–51° (I, R = CH_3), showed that it is different from both of them.

By the demethylation of gardenin, nor-gardenin has now been obtained in a pure condition and its analytical results agree with the requirements of a heptahydroxy-flavone. But in its properties it is very different from hibiscetin and 6-hydroxy-myricetin. We confirm that it does not give Bargellini's test but we should here mention that though this excludes the 5:6:7-disposition of hydroxyl groups, it does not rule out the 5:6:7:8-arrangement (II) as it has been shown recently by Seshadri and Venkateswarlu[5] that calycopteretin and its analogues having the 5:6:7:8- arrangement of hydroxyl groups do not give Bargellini's test. As a matter of fact this alternative had again to be reconsidered since the colour reactions of nor-gardenin towards alkaline buffer solutions were extraordinary. The absence of any display of colour so definitely associated with flavonols having hydroxyl groups in the 3′, 4′ and 5′ positions was remarkable. Actually not only hibiscetin[3] but its isomer 6-hydroxy-myricetin,[4] myricetin[6] itself (V, R = OH) and even robinetin[7] (V, R = H) give prominent display of colours in alkaline buffer solutions. This seemed to suggest that gardenin was a flavone derivative having the 5:6:7:8-arrangement of hydroxy groups because flavones generally do not give prominent colour changes in alkaline buffer solutions. However, as shown later, this constitution was eventually found to be untenable using synthetic evidence.

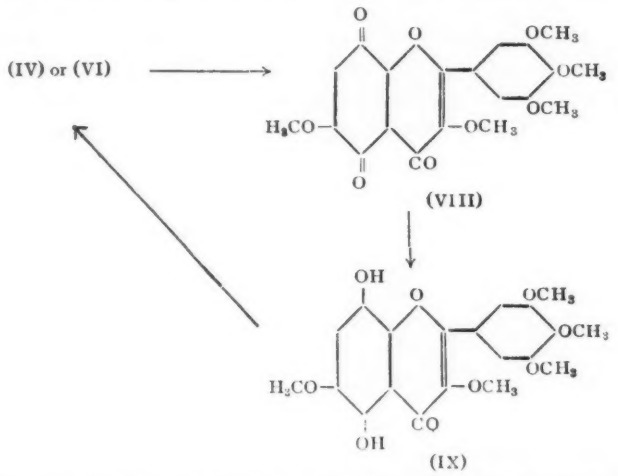
By carrying out the methylation of nor-gardenin in stages both gardenin and methyl gardenin could be obtained. This established that there was no isomeric change during the demethylation using hydriodic acid and also provided one method of preparing gardenin by partial methylation.

Alkali fission of gardenin itself does not proceed satisfactorily and produces further change in the ketonic fission product. On the other hand gardenin methyl ether (VI) undergoes fission smoothly with alcoholic potash and good yields of the ketonic part (VII) could be obtained along with trimethyl gallic acid. When these two are made to recombine using the Allan-Robinson method there is partial demethylation also and the product is found to be gardenin. These experiments confirm that gardenin belongs to the flavone group and also constitute a partial synthesis of gardenin itself.



A number of cases of partial demethylation during the Allan-Robinson condensation are now known leading to the formation of 5-hydroxy-compounds.

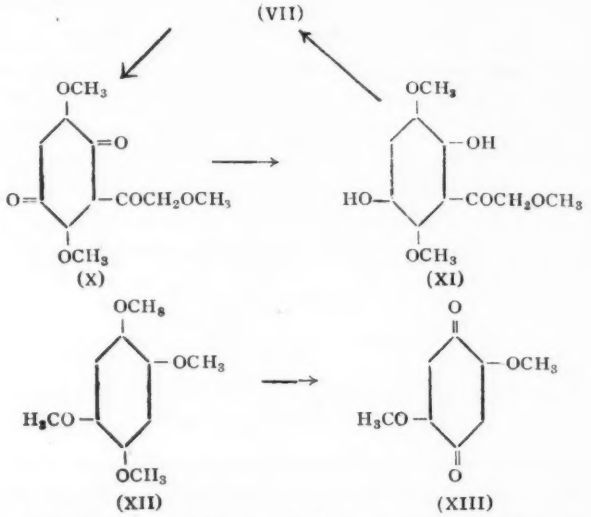
The behaviour of gardenin, as already recorded by previous workers, showed that it had a hydroxyl in the 5-position. Its preparation by the partial methylation of nor-gardenin and its partial synthesis and resistance to further methylation mentioned earlier in this paper agree with this structural feature. The boric-citric reaction[8] has now been found to be positive for the presence of the 5-hydroxyl in gardenin and 5-methoxyl in the methyl ether. With nitric acid not only gardenin (IV) but also its methyl-ether (VI) undergo change into the quinone gardeninone which is a definite indication of the presence of substituent groups in the 5 and 8 positions.[9] The quinone (VIII) could be reduced to the quinol (IX) and partial methylation of it yields again the original gardenin, whereas complete methylation yields the methyl ether. These transformations prove conclusively that nitric acid effects only oxidative demethylation and does not produce nuclear oxidation introducing fresh oxygen atoms into the nucleus. Further they constitute another easy method of preparing gardenin from its methyl ether and again confirm the location of the free hydroxyl in the 5-position.



The condensation of the fission ketone (VII) with benzoic anhydride is also accompanied by partial demethylation. But on further methylation a flavone tetramethyl ether is obtained with its melting point (118–19°) fairly close to the melting point of 5:6:7:8-tetramethoxy-flavone[10] (117–18°). But the mixed melting point is definitely lowered (95–100°). Consequently

the possibility of gardenin belonging to the 5: 6: 7: 8-hydroxy-flavone series (nobiletin series) is definitely ruled out and hence the only alternative left is that of a flavonol with the 5: 6: 8-arrangement of hydroxyls in the benzopyrone part. The high yield of the ketone in the fission and the facile reconstitution of gradenin would agree with the flavonol structure.

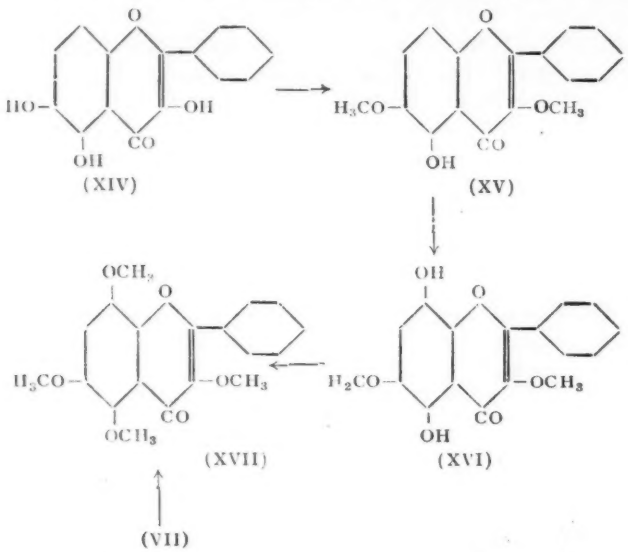
The following transformations carried out with the ketonic degradation product again agree with this idea and show definitely that it is ω-3: 5: 6-tetramethoxy-2-hydroxy-acetophenone (VII). On oxidation with nitric acid it underwent smooth oxidative demethylation yielding a quinone (X) which was neutral in properties and did not give ferric chloride colour. Estimation of methoxyl showed that it had still three methoxyls left. On reduction it formed a quinol (XI) which when partially methylated yielded the original tetramethoxy ketone. These transformations can be represented as given below.



A close analogy is provided by 1: 2: 4: 5-tetramethoxy benzene[11] (XII) which yields 2: 5-dimethoxy benzoquinone (XIII) on oxidation. In all the cases studied in the course of this work (gardenin and methyl gardenin, the fission ketone and tetramethoxy benzene) demethylation takes place only to form quinones and no extra methoxyl groups undergo removal during the process. These experiments on the ketone may be said to be complementary to those carried out on gardenin or its methyl ether. Whereas the latter give definite evidence about the presence of substituents in the 5: 8-positions, the former provide the proof for the presence of a methoxyl in

the 5-position of the ketone corresponding to the 6-position of the flavone. It may be mentioned here that with nitric acid the behaviour of 2-hydroxy-3:4:6-trimethoxy-acetophenone which has no methoxyl or hydroxyl group in the 5-position is different (unpublished work).

As already mentioned the condensation of the fission ketone (VII) with benzoic anhydride and sodium benzoate yields a compound which is different from 5:6:7:8-tetramethoxy-flavone. This led conclusively to the constitution of gardenin as a derivative of 3:5:6:8-hydroxy-flavone. Confirmation has now been obtained by comparing the above tetramethyl ether with a synthetic sample prepared by an independent route using nuclear oxidation.



The immediate starting point is 3:5:6-trihydroxy flavone (XIV) which has been recently described by Rajagopalan, Row and Seshadri.[12] It is partially methylated to yield the 3:6-dimethyl ether (XV) and subjected to oxidation with alkaline potassium persulphate. The quinol (XVI) obtained thereby is methylated when it yields 3:5:6:8-tetramethoxy flavone (XVII). In every respect it agrees with the tetramethyl ether obtained from gardenin-fission-ketone (VII) and the mixed melting point is undepressed.

The constitution of gardenin (IV,R = H) thus conclusively established, makes it the representative of a group of flavones till now unknown; the remarkable feature is that it is devoid of the 7-hydroxyl group. Compounds without this hydroxyl group are very few and are recent discoveries; primetin,

5-hydroxy-flavone, and flavone itself have so far been found to occur in nature. The marked absence of colour display in alkaline buffer solutions, already mentioned as characteristic of gardenin, is obviously bound up with the absence of this hydroxyl group since even the simpler flavonol, robinetin (V, R = H) gives this effect.

According to the theory of Robinson[13] regarding the biogenesis of anthocyanins and anthoxanthins, the 5:7-combination of hydroxyl groups in the benzopyrone part would represent the earliest stage. For the evolution of the 5:6:8-hydroxy structure as in gardenin there should be two further stages of oxidation involving the positions 6 and 8 and a stage of reduction involving position 7. Though it may not be possible to give the exact sequence of these processes the results already recorded on nuclear oxidation would suggest the following important alternatives.

$$\begin{array}{ccccc} 5:7 & \rightarrow & 5:6:7 & \rightarrow & 5:6:7:8 \\ \downarrow & & \downarrow & & \downarrow \\ \text{5-mono} & \rightarrow & 5:6 & \rightarrow & 5:6:8 \end{array}$$

The synthetic experiments described in this paper support the ready conversion of 5:6-dihydroxy compounds into 5:6:8-trihydroxy analogues. It may however be pointed out that the intermediate 5:6-dihydroxy compounds have not yet been found to occur in nature. As already indicated in an earlier paper[14] the 6-position should undergo oxidation during a stage of the skeleton when the pyrone ring is not closed whereas the 8-position is readily affected at a later stage when the pyrone ring is closed.

Experimental

Isolation of gardenin (IV, R = H)

The following procedure, for the extraction of Dikamali gum, was found to give consistently good yields of gardenin.

The gum was repeatedly digested with small volumes of boiling alcohol until the alcoholic extract was no more coloured yellow. Each time the clear supernatant liquid was decanted off quickly while hot. The total alcoholic extract was left in the refrigerator for one week. Almost all the gardenin along with some yellow resin separated out. This was filtered through fluted filters. The solid on the filter was now thoroughly shaken with hot petrol when all the resinous part went into solution. The residue was filtered and crystallised twice from alcohol. It was obtained as golden yellow needles melting at 163–64°. (Found: C, 59·8; H, 4·9; OCH_3, 44·5; $C_{21}H_{22}O_9$ requires C, 60·3; H, 5·3; OCH_3, 44·5%.) With alcoholic

ferric chloride gardenin gave an olive green colour and in aqueous alkali it was very sparingly soluble. When an alcoholic solution was acidified with hydrochloric acid and treated with magnesium powder an orange red colour was readily produced. It gave the boric-citric test characteristic of a 5-hydroxyl in the flavone nucleus.

Nor-gardenin

Gardenin (1·0 g.) was dissolved in acetic anhydride (5·0 c.c.) and hydriodic acid (10 c.c.; d, 1·7) was added with cooling under the tap. The mixture was boiled gently for one hour, cooled and diluted with water and saturated with sulphur dioxide. The bright yellow solid that separated out was crystallised from glacial acetic acid. It was obtained as micro-crystalline solid melting with decomposition at 320°. (Found: C, 51·5; H, 3·7; $C_{15}H_{10}O_9$, H_2O requires C, 51·1; H, 3·4%.) Yield, 0·6 g.

With alcoholic ferric chloride it gave a green colour which changed to blue and later gave rise to a blue precipitate. In aqueous alkali it dissolved quickly to form a reddish-brown solution. With *p*-benzoquinone in glacial acetic acid the compound produced a bright red colour followed by a reddish brown precipitate. It gave the following colour changes in buffer solutions of different p_H: p_H 8·2—light green solution changing rapidly to yellow; p_H 9·2—dissolved to give a yellow solution, no change in 24 hours; p_H 10·4—formed a deep yellow solution and turned yellowish-brown in 24 hours; p_H 11·6—golden-yellow solution slowly acquiring brown tinge and becoming pale brown red, in 24 hours changed to yellowish-brown; p_H 12·8—orange solution, brown in 2 minutes and yellowish-brown in 24 hours; sodium carbonate solution—dull green, fading rapidly to yellowish brown. With lead acetate solution it gave a red precipitate changing to brown and with sodium amalgam and absolute alcohol a brown solution followed by a brown precipitate.

Methyl gardenin (VI)

Gardenin (1·0 g.) was dissolved in dry acetone (50 c.c.) and anhydrous potassium carbonate (8·0 g.) and dimethyl sulphate (0·5 c.c.) were added. The mixture was refluxed for 30 hours. The acetone solution was filtered from the potassium salts which were washed with some more hot acetone. The acetone filtrate on concentration and dilution with water gave a colourless solid. It crystallised from a mixture of dry ethyl acetate and petroleum-ether as colourless stout rectangular rods melting at 116–17°. (Found: C, 61·4; H, 5·5; OMe, 49·6; $C_{22}H_{24}O_9$ requires C, 61·1; H, 5·6; OMe, 50·2%.) Yield 0·8 g. The compound was perfectly colourless and

insoluble in aqueous alkali and produced no colour with alcoholic ferric chloride. It gave a positive boric-citric reaction.

Complete methylation of nor-gardenin

Nor-gardenin (0·5 g.) was methylated in dry acetone (50 c.c.) using excess of dimethyl sulphate (1 c.c.) and anhydrous potassium carbonate (8·0 g.). The methyl ether, after a crystallisation from a mixture of dry ethyl acetate and petroleum-ether, was obtained as a colourless crystalline solid. The mixed melting point with methyl gardenin (VI) was undepressed.

Partial methylation of nor-gardenin

Nor-gardenin (0·2 g.) was methylated in dry acetone (25 c.c.) using dimethyl sulphate (0·28 c.c., 6 mols.) and anhydrous potassium carbonate (5·0 g.). The methylated product crystallised out of alcohol as bright yellow needles melting at 162–63°. The mixed melting point with gardenin (IV) was not depressed.

Gardeninone (VIII) and Gardeninol (IX)

Following the procedure of Bose and Nath[2] gardeninone (m.p. 222°) was prepared from gardenin and also from methyl gardenin using nitric acid (d, 1·25) and it was reduced to gardeninol. The quinol was found to melt at 190–91° as recorded by Stenhouse. With alcoholic chloride it gave a green colour which immediately changed to brown and it was soluble in aqueous alkali to form a reddish-brown solution.

Complete methylation of gardeninol (IX)

Gardeninol (IX) (0·5 g.) was dissolved in dry acetone (25 c.c.) and anhydrous potassium carbonate (8·0 g.) and dimethyl sulphate (0·8 c.c.) were added. The mixture was refluxed for 30 hours and on working up the acetone solution as in the methylations described already a colourless compound melting at 116–17° was obtained. The mixed melting point with methyl gardenin (VI) was undepressed.

Partial methylation of gardeninol

Gardeninol (IX) (0·3 g.) was dissolved in dry acetone (25 c.c.) and anhydrous potassium carbonate (5·0 g.) and dimethyl sulphate (0·08 c.c.; 1 mol.) were added. The mixture was refluxed for six hours and the acetone solution worked up as usual. The bright yellow solid product melted at 163–64° after crystallisation from alcohol. The mixed melting point with gardenin (IV) was not depressed.

Alkali fission of methyl gardenin (VI)

Methyl gardenin (VI) (1·0 g.) was treated with 8% absolute alcoholic potash (30 c.c.) and the solution was refluxed for six hours. As much of the alcohol as possible was removed by evaporation over a water-bath and the residue was treated with water. The solution was filtered through cotton and acidified with dilute sulphuric acid. The semi-solid that separated out was extracted with ether. The ether solution was repeatedly extracted with aqueous sodium bicarbonate. On acidifying this extract a colourless solid was precipitated which, when crystallised from hot water, was obtained as colourless needles melting at 167–68°. The mixed melting point with an authentic sample of trimethyl gallic acid was not depressed.

The ether solution on evaporation deposited a bright yellow semi-solid which solidified slowly. It crystallised from alcohol as bright yellow cubes (VII) melting at 88–89°. (Found: C, 56·0; H, 6·5; OCH_3, 47·9; $C_{12}H_{16}O_6$ requires C, 56·3; H, 6·3; OCH_3, 48·4%.) Yield, 0·5 g. With alcoholic ferric chloride it gave a reddish-brown colour and was soluble in aqueous alkali. Direct comparison and mixed melting point determination showed that it is different from gossypetol and quercetagetol-tetramethyl ethers.

Oxidation of the ketone (VII)

The ketone (2·0 g.) was dissolved in dry ether (150 c.c.) and fuming nitric acid (1·0 c.c.) was added. On stirring the mixture and allowing it to stand for half an hour, a bright red solid (quinone X) separated out. It was filtered and washed with ether. It crystallised from dilute acetic acid as long orange-coloured needles melting at 222–24°. (Found: OCH_3, 38·4; $C_{11}H_{12}O_6$ requires OCH_3, 38·8%.) Yield, 0·8 g. With alcoholic ferric chloride it did not give any colour. It was insoluble in sodium carbonate solution but dissolved slowly in aqueous sodium hydroxide. It liberated iodine from an acidified solution of potassium iodide.

Reduction of the quinone (X)

The quinone (X) (0·8 g.) was suspended in rectified spirit (200 c.c.) and the mixture saturated with sulphur dioxide and left overnight. The alcoholic solution was filtered and concentrated when a bright yellow solid separated out. It crystallised out of alcohol as bright yellow needles melting at 175–77°. (Found: OMe, 38·6; $C_{11}H_{14}O_6$ requires OMe, 38·4%.) With alcoholic ferric chloride it gave a green colour changing to brown and it dissolved in alkali to a reddish-brown solution.

Methylation of the quinol (XI)

The quinol (XI) (0·2 g.) was methylated in dry acetone medium (25 c.c.) using dimethyl sulphate (0·1 c.c.; 1 mol.) and anhydrous potassium carbonate (5 g.). The partially methylated compound crystallised from alcohol as a bright yellow crystalline solid melting at 87–88°. The mixed melting with the original ketone (VII) was not depressed.

Condensation of the ketone (VII) with the anhydride and sodium salt of trimethyl gallic acid.

An intimate mixture of the ketone (VII) (1·0 g.), trimethyl gallic anhydride (10·0 g.) and sodium salt of trimethyl gallic acid (4·0 g.) was heated at 175–80° under reduced pressure for three hours. The product was dissolved in boiling alcohol (50 c.c.) and an aqueous solution of potassium hydroxide (6·0 g. in 8 c.c. of water) was added to it. The mixture was boiled for 15 minutes and the alcohol was removed under reduced pressure. The residue was dissolved in water. When the aqueous solution was saturated with carbon dioxide, a bright yellow solid separated out which, after recrystallisation from alcohol, melted at 161–62°. Yield, 0·6 g. The mixed melting point with gardenin (IV) was undepressed. The condensation product was methylated in dry acetone medium using dimethyl sulphate and anhydrous potassium carbonate. The methyl ether, after a crystallisation from dry ethyl acetate, melted at 116–17°. The mixed melting point with methyl gardenin (VI) was undepressed.

Condensation of the ketone (VII) with benzoic anhydride and sodium benzoate

The ketone (1·0 g.) was intimately mixed with benzoic anhydride (10·0 g.) and sodium benzoate (4·0 g.) and the mixture was heated at 175–80° under reduced pressure for three hours. The product was worked up as before, and was obtained as a pale yellow solid (0·6 g.). It gave a green colour with alcoholic ferric chloride and was slightly soluble in aqueous alkali. It was directly methylated using dimethyl sulphate and anhydrous potassium carbonate in dry acetone medium. The tetramethyl ether (XVII) crystallised out of a mixture of ethyl acetate and petroleum-ether as stout rectangular prisms melting at 118–19°. (Found: C, 66·5; H, 5·0; $C_{19}H_{18}O_6$ requires C, 66·7; H, 5·3%.) Yield, 0·4 g. With alcoholic ferric chloride it did not give any colour and was insoluble in aqueous alkali. The mixed melting point with 5:6:7:8-tetramethoxy-flavone (m.p. 117–18°) was considerably depressed (95–100°).

3 : 6-*Dimethoxy*-5-*hydroxy-flavone* (*XV*)

The trihydroxy-flavone (XIV)[12] (1·0 g.) was dissolved in dry acetone (50 c.c.) and anhydrous potassium carbonate (10 g.) and dimethyl sulphate (0·7 c.c.) were added. The mixture was refluxed for six hours and the acetone solution was filtered off from the potassium salts. After concentrating the acetone solution, the product was crystallised from alcohol. It was obtained as pale yellow rectangular plates melting at 108–109°. (Found: C, 68·2; H, 5·0; $C_{17}H_{14}O_5$ requires C, 68·5; H, 4·7%.) With alcoholic ferric chloride it gave an olive-green colour and was sparingly soluble in aqueous alkali.

3 : 6-*Dimethoxy*-5 : 8-*dihydroxy flavone* (*XVI*)

The 5-hydroxy compound (XV) (1·0 g.) was dissolved in pyridine (20 c.c.) and aqueous sodium hydroxide (0·6 g. in 15 c.c. of water) was added. To the mixture an aqueous solution of potassium persulphate (1·5 g. in 30 c.c. of water) was added gradually drop by drop while the solution was stirred continuously. The solution was kept overnight and made just acid to congo-red with hydrochloric acid. The unreacted compound that separated out was filtered off and the filtrate extracted with ether to remove the last traces. Sodium sulphite (3 g.) and concentrated hydrochloric acid (20 c.c.) were added to the aqueous solution and the mixture was heated on a boiling water-bath for half an hour. The solid that separated out was filtered and crystallised from alcohol. The quinol (XVI) was thus obtained as bright yellow needles melting at 185–86°. (Found: C, 64·6; H, 4·9; $C_{17}H_{14}O_6$ requires C, 65·0; H, 4·5%.) With alcoholic ferric chloride it gave a green colour which changed to brown and it was freely soluble in aqueous alkali producing a red solution.

3 : 5 : 6 : 8-*Tetramethoxy-flavone* (*XVII*)

The 5 : 8-dihydroxy compound (XVI) (0·2 g.) was dissolved in dry acetone (25 c.c.) and anhydrous potassium carbonate (5 g.) and dimethyl sulphate (0·5 c.c.) were added. The mixture was refluxed for 30 hours and the acetone solution was worked up as in similar cases. The tetramethyl ether crystallised from ethyl acetate as stout rectangular prisms melting at 117–18°. (Found: C, 66·9; H, 5·7; $C_{19}H_{18}O_6$ requires C, 66·7; H, 5·3%.) It gave no colour with alcoholic ferric chloride and was insoluble in alkali. The mixed melting point with the tetramethoxy compound obtained from the fission ketone (VII) was undepressed.

SUMMARY

The constitution suggested by Bose for gardenin (5-hydroxy-3 : 6 : 8 : 3′ : 4′ : 5′-hexamethoxy-flavone) has been confirmed in the following manner.

(1) Preparation of nor-gardenin and gardenin methyl ether and comparison with hibiscetin and 6-hydroxy-myricetin and their methyl ethers. There was no agreement.

(2) Oxidative demethylation of gardenin and its methyl ether. This proved that groups exist in the 5 and 8 positions.

(3) Alkali fission of methyl gardenin and study of the ketonic product. It condensed with the anhydride and sodium salt of trimethyl gallic acid to reform gardenin. Its oxidation with nitric acid and subsequent transformations showed that it has a methoxyl in the 5-position corresponding to the 6-position in gardenin.

(4) The tetramethoxy flavone obtained from the fission ketone and benzoic anhydride and sodium benzoate was not identical with 5:6:7:8-tetramethoxy-flavone, but was identical with 3:5:6:8-tetramethoxy-flavone synthetically obtained by the method of nuclear oxidation.

(5) Gardenin could be directly obtained by the partial methylation of nor-gardenin or gardeninol and by the Allan-Robinson condensation using the fission ketone and trimethyl gallic acid.

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CONSTITUTION OF HIBISCITRIN. PART I

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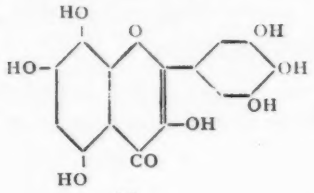
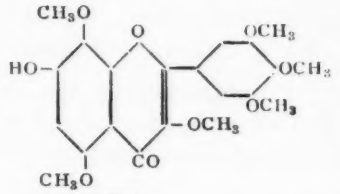
In an earlier paper[1] the isolation of a new flavonol glucoside, hibiscitrin from the flowers of *Hibiscus sabdariffa* was described. The constitution of the aglucone, hibiscetin was also established as 3 : 5 : 7 : 8 : 3′ : 4′ : 5′-heptahydroxy-flavone (I) and later confirmed by synthesis.[2] The exact constitution of hibiscitrin has subsequently been followed up and the results are reported in this paper.



(I)



(II)

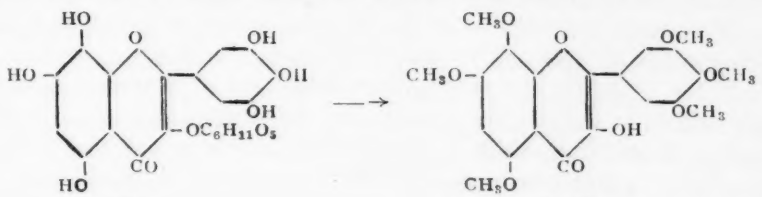
Quantitative study of the hydrolysis has shown that hibiscitrin yields a molecular proportion each of hibiscetin and glucose thus showing that it is a mono-glucoside. The other analytical data have therefore been re-examined. C and H values for the air-dried sample originally recorded and also ob ained on repeating the analysis are found to agree with a dihydrate formula of the monoglucoside. Special difficulty has been experienced in drying the substance and the results relating to the anhydrous compound have not been satisfactory.

There were originally difficulties in locating the position of the glucose group. The acetate of hibiscitrin could not be obtained pure and crystalline and hence methylation through the acetate could not be adopted.[3] Methylation using diazomethane was next attempted. Though this reagent does not effect complete methylation in the case of 5 : 7 : 8-hydroxy-flavonols and their derivatives[4] even when employed in large excess it was hoped that at least partial methylation would take place and that the process could be completed by other methods. But no appreciable reaction took place owing mostly to the sparing solubility of the glucoside in cold alcohol-ether mixture in which the reaction had to be carried out. Since hibiscitrin gave the gossypetone reaction readily, it could be surmised that the hydroxyl

groups in the 5- and 8-positions were free. But even this was likely to be in error since the pyrogallol side-phenyl nucleus is also capable of giving this reaction as noted in the case of myricetin.[5] The colour reactions of hibiscitrin in alkaline buffer solutions showed considerable resemblance to those of gossypitrin and herbacitrin, but there was also some marked difference.

Eventually it was found possible to methylate hibiscitrin fully by boiling an acetone suspension of it with excess of dimethyl sulphate and anhydrous potassium carbonate. The glucoside got slowly methylated and the fully methylated product was finally formed. The completion of methylation was indicated by the absence of the ferric chloride colour. The methylated glucoside could not be obtained crystalline, but on hydrolysis yielded a hexamethyl hibiscetin (A) having only one hydroxyl group left free. The formation of the monohydroxy compound agreed with the observation that hibiscitrin is a monoglucoside.

Alkali fission of the hexamethyl hibiscetin (A) yielded trimethyl gallic acid. This showed that the side-phenyl nucleus did not contain the free hydroxyl and hence was not involved in the linking of the sugar. In this experiment the ketonic fission product could not be isolated for study owing to further decomposition. It was however definite that the benzopyrone part of the molecule carried the hydroxyl group. Compound (A) was different from the already known hexamethyl hibiscetin (II) with the 7-hydroxyl free which had been prepared as an intermediate stage in the earlier synthesis of hibiscetin.[2] The latter has a higher melting point 238–40°, whereas the former melts at 198–200°. Further compound (A) is deep yellow in colour and gives a prominent brown colour with alcoholic ferric chloride. A single free hydroxyl group in the 7- or 8-position or in the side-phenyl nucleus cannot give rise to these properties. Only the 3- or 5-position could be involved. The latter is improbable since it is comparatively unreactive and does not get easily substituted. On the other hand the 3-hydroxyl is quite reactive and 3-glucosides are quite common. Hence hibiscitrin should be considered to be a 3-monoglucoside (III). In agreement with this, the degradation product (A) which should be given the structure (IV) is readily soluble



(III) (IV) (A)

in aqueous alkali. Flavone derivatives with only one hydroxyl group in the 5-position are only very sparingly soluble in aqueous alkali and form sparingly soluble salts.

Herbacetin, gossypetin and hibiscetin are three flavonols with the 5:7:8-arrangement of hydroxyl groups and occurring in nature predominantly as glucosides. Of these, herbacitrin and gossypitrin are 7-glucosides[3] resembling each other closely in their properties and reactions and they occur together in the petals of the Indian cotton flowers.[6] Gossypin is an 8-glucoside[7] of gossypetin and it differs markedly from the abovementioned 7-glucosides in several important respects. On the other hand, the 3-glucoside, hibiscitrin does not appear to exhibit so much difference. Its reactions with ferric chloride and *p*-benzoquinone are just similar to those of gossypitrin and herbacitrin. Even in regard to the colour reactions in alkaline buffer solutions there is considerable resemblance. But hibiscitrin is less sensitive to the solutions of lower p_H and gives the best effect at higher p_H; with gossypitrin and herbacitrin the position is just the reverse. Further as the final colour hibiscitrin yields a prominent red whereas the 7-glucosides yield only a pale brown colour.

Experimental

The extraction of the flowers and the isolation of hibiscitrin were much quicker by adopting the following procedure.

The dried flowers (500 g.) were extracted twice with boiling alcohol, each extraction taking 6 hours. The combined extract was concentrated to about 100 c.c. by distillation on a water-bath. On cooling, a good amount of wax and resin separated out and they were removed by filtration using fluted filters. The clear filtrate was diluted with a large volume of water and the remaining alcohol evaporated and the solution concentrated on a water-bath. During this operation some more resin separated and was removed by filtration through cotton. The clear filtrate was then saturated with ether and allowed to stand. A yellow crystalline solid began to separate out slowly and the process was complete in about a week. It was filtered and crystallised twice from alcohol when it came out as yellow rectangular plates melting at 238–40° (decomp.). (Found in air-dried sample: C, 47·5, 47·4; H, 4·7, 4·4; $C_{21}H_{20}O_{14}$, $2H_2O$ requires C, 47·4; H, 4·5%.) Hibiscitrin is sparingly soluble in water, ether, acetone and cold alcohol and is more soluble in boiling alcohol from which it crystallises out slowly on cooling. In alcoholic solution it gives rise to an olive brown colour with ferric chloride and a deep orange red precipitate with neutral lead acetate. It dissolves in

concentrated sulphuric acid to form an orange red solution with no fluorescence. A deep reddish brown quinone is quickly precipitated by treating the substance with benzoquinone in alcoholic solution. The colour changes exhibited by the glucoside with alkaline buffer solutions are recorded below:

p_H 8·6.—Yellow solution slowly assuming a brownish yellow colour; changes to pale orange (10 min.); after 20 minutes orange red, stable for 24 hours.

p_H 9·2.—Deep yellow becoming greenish yellow (½ min.); green rapidly disappears (1 min.) and brown tinge appears intensifying to bright brown red within 10 minutes; after 20 minutes orange red stable for 24 hours.

p_H 9·8.—Deep yellow changing to green (½ min.); after 1½ minutes green fading to yellowish green; brownish yellow in 3 minutes. After 5 minutes, brown red changing to bright red (10 minutes), stable for 24 hours.

p_H 10·4.—Deep yellow changing to emerald green immediately; changes to reddish brown (2½ min.), deep brownish red (5 min.) and bright red (10 min.) stable for 24 hours.

p_H 11·0.—The same colour changes but faster; in 4 minutes red, stable for 24 hours.

p_H 12·2.—Initial deep yellow changes to emerald green, greenish violet, reddish violet, red (4 minutes) stable for 24 hours.

p_H 12·8.—Deep yellow immediately changing to emerald green; on shaking, deep bluish green and finally deep red (3 minutes) stable for 24 hours.

p_H 13·4.—Very rapid succession of colours; green, emerald green, bluish green, slate colour, reddish brown and red within 2 minutes.

Sodium bicarbonate.—Yellow changing to orange red and then bright red, stable for 2 hours. Pale orange after 24 hours.

5% sodium carbonate.—Deep emerald green changing on shaking with air to deep blue (1 min.) and to violet (2½ min.), pale orange (10 min.) stable for 24 hours.

2% sodium hydroxide.—Green colour immediately changing to blue; on shaking, a bluish brown colour is obtained (2 min.); changes to brownish violet (3 min.) and orange red (4 min.) stable for 24 hours.

In contrast with the above, gossypitrin gives the following reactions:

Sodium bicarbonate (saturated solution).—Partially soluble, slowly assuming pale green and green; pale brown after 24 hours.

5% sodium carbonate.—Completely soluble; pale green slowly turning yellowish brown and then brown fading to very pale brown.

2% sodium hydroxide.—Deep brownish red, changes to pale red after 3 minutes, stable for 24 hours.

Hydrolysis of Hibiscitrin

Hibiscitrin (1·0 g.) was boiled under reflux with 7% sulphuric acid (100 c.c.) for two hours. It was rapidly hydrolysed and in about half an hour there was clear solution and the aglucone began to separate out. At the end of the experiment the solid was filtered off and the filtrate extracted with ether so as to remove all the aglucone. The total amount of hibiscetin thus obtained was dried in air and then weighed. The filtrate was neutralised using first sodium hydroxide and then sodium carbonate, clarified with neutral lead acetate and the sugar was estimated gravimetrically by the method of Munsen and Walker. The result was verified by volumetric titration with standard Fehling's solution using methylene blue as indicator. [Found: aglucone, 65·5; glucose, 34·1; $C_{21}H_{20}O_{14}$, $2H_2O$ requires aglucone (monohydrate), 66·2%; glucose 33·8%.] The sugar was identified as glucose by preparing its osazone and the aglucone was found to be identical with an older sample of hibiscetin.

Hibiscetin methyl ether

As the older method of methylation using the aglucone acetate, sodium hydroxide and dimethyl sulphate was not convenient and did not give good yields, the following procedure was adopted. Hibiscetin (2 g.) was dissolved in anhydrous acetone (50 c.c.); the solution was treated with anhydrous potassium carbonate (25 g.) and dimethyl sulphate (8 c.c.) and the mixture boiled under reflux for 30 hours. A further quantity of dimethyl sulphate (6 c.c.) was added in small quantities during the first 24 hours. The mixture was finally filtered under suction while still hot and the residue was washed with small quantities of hot anhydrous acetone. The filtrate was concentrated and on the addition of excess of water a bulky precipitate of the methyl ether was produced. It crystallised from dilute acetic acid as shining colourless needles and narrow rectangular plates melting at 194-96°. Its crystal structure and melting point were identical with those of the hepta-methyl ether already described.

Alkali fission of the Heptamethyl hibiscetin

This experiment was not reported earlier. Heptamethyl hibiscetin (1·0 g.) was treated with absolute alcoholic potash (2·5 g. of potash in 30 c.c. of absolute alcohol) and boiled under reflux on a water-bath for about six hours. As much of alcohol as possible was then removed by distillation, the residue dissolved in water and the solution filtered to remove impurities. The clear filtrate was acidified with excess of dilute sulphutric acid. The product was then ether-extracted and the ether solution was washed repeatedly with 5% sodium bicarbonate solution till no more acid could be extracted. The ether solution was then distilled to remove the solvent and the solid obtained was recrystallised from dilute alcohol. It appeared as colourless flat needles melting at 115–16° and the mixed melting point with an authentic sample of gossypetol tetramethyl ether was undepressed.

The sodium bicarbonate washings were acidified with concentrated hydrochloric acid when a small amount of precipitate separated out. The mixture was ether-extracted and the product was recrystallised from hot water. The acid was found to be identical with a synthetic sample of trimethyl gallic acid.

Methylation of Hibiscitrin and hydrolysis

Hibiscitrin (2 g.) was suspended in anhydrous acetone (50 c.c.) and the mixture was refluxed for 30 hours after adding freshly distilled dimethyl sulphate (6 c.c.) and anhydrous potassium carbonate (15 g.). The mixture was finally filtered under suction while still hot and the residue was washed with a small quantity of hot anhydrous acetone. The filtrate was concentrated to remove as much of acetone as possible. The residue was a viscous semi-solid and it could not be crystallised even after repeated attempts. Therefore it was hydrolysed by boiling with 200 c.c. of 7% sulphuric acid for two hours. A clear solution was first obtained and then a deep yellow solid began to separate out at the end of the first half hour giving rise to bumping. The product was filtered; a further yield of it could be obtained by thoroughly ether-extracting the filtrate. It was recrystallised from dilute alcohol from which it separated out as bright yellow short needles melting at 198–200°. (Found: C, 59·9; H, 5·2; $C_{21}H_{22}O_9$ requires C, 60·3; H, 5·3%.)

The hexamethyl ether (A) was soluble in alkali to form a reddish brown solution and gave a dark brown colour with ferric chloride in alcoholic solution. An alcoholic solution of the substance did not give any precipitate or colour with either neutral or basic lead acetate. This solution exhibited no fluorescence. A solution in concentrated sulphuric acid was deep yellow without fluorescence.

Alkali fission of hexamethyl hibiscetin (A)

Hexamethyl hibiscetin (A) from Hibiscitrin was boiled under reflux with absolute alcoholic potash (30 c.c.; 8%) for a period of six hours. At the end of the experiment as much of alcohol as possible was removed by distillation and the residue was dissolved in water. The clear solution was acidified and extracted with ether. The ether solution was extracted repeatedly with 5% sodium bicarbonate solution until all the acid was removed. The bicarbonate washings were acidified and the precipitated acid was recrystallised from hot water from which it was obtained as colourless rectangular plates melting at 167–68°. It was identified as trimethyl gallic acid. The mixed melting point with an authentic sample of trimethyl gallic acid was undepressed. The remaining ether solution did not yield any definite product perhaps due to further decomposition of the ketonic part.

Summary

Analysis and study of acid hydrolysis show that hibiscitrin is a monoglucoside of hibiscetin. It undergoes complete methylation with dimethyl sulphate and anhydrous potassium carbonate in anhydrous acetone medium; on hydrolysing the methyl ether a O-hexamethyl hibiscetin is obtained which yields trimethyl gallic acid on fission with alkali. From this reaction and from other characteristic properties of the above partial methyl ether it is concluded that the free hydroxyl is in the 3-position and that hibiscitrin is a 3-glucoside.

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CHEMICAL EXAMINATION OF PLANT INSECTICIDES

Part V. A Comparative Study of Scandenin, Lonchocarpic Acid and Robustic Acid

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Among the crystalline non-rotenoid compounds occurring in leguminous plants scandenin, lonchocarpic acid and robustic acid seem to be the most important. They can be obtained in adequate quantities for study and seem to have an appreciable amount of toxicity though poor when compared with rotenone and related rotenoids. But the interesting point is that besides the negative Durham test they appear to have a number of other properties in common and to be closely related in their molecular structures thus constituting a type of chemical compounds. As a matter of fact Clark[1] claimed to have found all the three occurring together in the roots of American *Derris scandens* though this finding could not be supported from our study of the Indian material.[2] However between the individuals there are differences. These features have now been investigated more closely and are discussed in this paper.

Scandenin, the chief chemical component of the roots of *Derris scandens*, was first isolated by Clark[1] and with the small quantity of material available to him a brief chemical study was made. It was given the molecular formula $C_{26}H_{26}O_6$ and was found to have one methoxyl and two free hydroxyl groups capable of forming a diacetate and a dimethyl ether. It was acidic in character and produced sparingly soluble sodium and potassium salts. As the acetate was insoluble in aqueous alkali he concluded that the acid nature of the substance was not due to a carboxyl but due to the presence of an active hydroxyl group. The compound showed considerable unsaturation and absorbed three molecular proportions of hydrogen, though no definite hydrogenation products were isolated. The probability of the presence of *p*-hydroxy-benzoyl group in scandenin was indicated by the formation of *p*-hydroxy-benzoic acid on oxidation with alkaline hydrogen peroxide. No oxime or semicarbazone could be prepared. The substance did not give the Durham test. From these properties Clark concluded that scandenin bore no relationship to the rotenone group of fish poisons.

Since we could isolate a fairly good quantity of scandenin during the course of our examination[2] of *D. scandens* obtained from Central India, a more detailed study has now been carried out. The compound is definitely toxic to fish though very inferior to rotenone. It is optically inactive just like lonchocarpic and robustic acids.

The molecular formula, $C_{26}H_{26}O_6$ has now been confirmed. That scandenin has a minimum molecular size of 420 could be deduced from its methoxyl content, the titration with alkali and the estimation of potassium in the sparingly soluble potassium salt. Determination of the molecular weights of scandenin and of its methyl ether using Rast's method established that the molecular weight of scandenin is of the order 420 and not a multiple of it. The compound thus behaves like a monobasic acid and the salt is a monopotassium salt.

Scandenin is not only soluble in alkali but as mentioned above it could actually be titrated with alkali. However it is a weak acid and is thrown out of an alkaline solution by passing carbon dioxide. Besides its acetate being insoluble in alkali, its dimethyl ether does not undergo hydrolysis like an ester. Further scandenin is unaffected under conditions of esterification. These are in conformity with the suggestion of Clark that the acid property is due not to a carboxyl group but to an active hydroxyl group. We confirm that scandenin does not give ferric chloride colour and that it forms *p*-hydroxybenzoic acid readily on oxidation with alkaline hydrogen peroxide. We have also been unable to get derivatives indicative of the presence of a carbonyl group; no oxime or dinitrophenyl hydrazone could be prepared. But the study of the ultra-violet absorption spectrum mentioned later seems to indicate the presence of a carbonyl group. This is also supported by the toxic properties of the compound since most plant insecticides contain this group.

Clark[1] prepared the diacetate by boiling scandenin for 5 minutes with acetic anhydride and sodium acetate. As reported in Part II[2] by boiling for a longer period a purer product is obtained in better yield. Deacetylation with alcoholic potash regenerates scandenin. For purposes of methylation Clark used diazomethane and could obtain only poor yields of the dimethyl ether. By employing dimethyl sulphate and anhydrous potassium carbonate in dry acetone medium very good yield of the pure dimethyl ether could now be readily produced.[2] Methylation with dimethyl sulphate and aqueous alkali forms the same product, but the yield is less. Attempts to isolate a monomethyl ether by employing one molecular proportion of dimethyl sulphate have been unsuccessful; only a mixture of the dimethyl ether and the potassium salt of scandenin are obtained.

Scandenin dissolves in concentrated sulphuric acid in the cold producing an intense orange red colour without any fluorescence. When the solution is poured on ice it gives a sulphonated product which does not melt below 270°; it is fairly soluble in alcohol, acetone and water, but insoluble in ether. In anqueous solution it gives a pure blue colour with ferric chloride. Attempts to crystallise it have not been successful. The experiment indicates that in cold sulphuric acid no simple isomerisation takes place and that the molecule of scandenin undergoes sulphonation readily.

Scandenin is found to be extraordinarily stable to alkali, boiling with alcoholic alkali, aqueous alcoholic alkali and even 50% aqueous potash producing no change. Almost all the scandenin could be recovered unchanged after this treatment. The potassium salt that is formed seems to be quite stable.

In order to get some idea of the nature of unsaturation in scandenin the reaction of bromine has been studied. Scandenin readily forms a well-defined bromo-derivative in acetic acid medium. The reaction seems to take place without elimination of hydrobromic acid and the bromo-derivative could therefore be taken to be an addition product. Its analysis indicates that it is a dibromide. In order to make sure that nuclear bromination is not involved owing to the influence of the phenolic hydroxyl groups, the dimethyl ether and the diacetate have also been subjected to treatment with bromine. They also behave similarly taking up two bromine atoms. It appears therefore that in all these reactions an ethylenic double bond is involved.

Lonchocarpic acid was first isolated by Jones[3] from an unknown species of Lonchocarpus from Venezula and was further studied by Jones and Haller.[4] It was found to have the formula $C_{26}H_{28}O_6$, to be soluble in sodium carbonate and stable to alcoholic alkali. Though at first it was considered to be a carboxylic acid the more recent work showed that the acid properties are due to a strongly acidic hydroxyl group only. Like scandenin, this also has one methoxyl and two hydroxyl groups. The diacetate is insoluble in aqueous alkali. Lonchocarpic acid formed both mono and dimethyl ethers which did not exhibit the properties of esters. Catalytic hydrogenation added 4 hydrogen atoms and a tetrahydro derivative could be prepared. Oxidation of lonchocarpic acid with alkaline hydrogen peroxide formed *p*-hydroxybenzoic acid.

In order to identify the crystalline substances isolated from *D. scandens*, a sample of lonchocarpic acid was obtained from Dr. H. A. Jones and a number of its derivatives prepared, We had also occasion to study its

properties and compare them with those of scandenin. They resemble in several respects. They have the same molecular formula, have one methoxyl and two hydroxyl groups in their molecules and thus form diacetates and dimethyl ethers. They are acidic in nature and are quite stable to alkali. Even in the hydrogen peroxide oxidation they behave alike forming *p*-hydroxybenzoic acid. They are also optically inactive.

Jones[3] reported that lonchocarpic acid had no insecticidal properties. In our tests using fresh-water fish it exhibited definite toxic properties. Though it is slower in its effect than even scandenin there is one noteworthy feature. The fish recovered when transferred to fresh water after the experiment in the case of scandenin as with most substances. But with lonchocarpic acid they all died. This showed that lonchocarpic acid is quite toxic though it may be slow acting.

The differences that we could so far note in their properties are as follows: Lonchocarpic acid is more easily soluble in alcohol than scandenin; it is also soluble in 5% sodium carbonate solution whereas scandenin forms a sparingly soluble salt. With concentrated sulphuric acid the former gives a deeper red solution. There is a small difference in the colour changes shown by the two substances under the Durham test.

Just as in the case of scandenin chemical tests for the presence of a carbonyl group are not given by lonchocarpic acid also. But the presence of such a group is indicated by the ultra-violet absorption spectrum and the agreement between the spectra of the two compounds is very close.

Even in regard to bromine addition there is resemblance between scandenin and lonchocarpic acid. The dimethyl ether and diacetate have been treated with excess of bromine in acetic acid solution. There is no elimination of hydrogen bromide and two atoms of bromine are found in the addition products.

In the course of their examination of the roots of *Derris robusta* Rao and Seshadri[6] made a preliminary study of robustic acid. They showed that its molecular formula is $C_{22}H_{20}O_6$ and thus it has 6 oxygen atoms just as scandenin and lonchocarpic acid. It is a dimethyl ether having a free hydroxyl group which is capable of acetylation and methylation. Thus the fully methylated ether has three methoxyl groups just as those of scandenin and lonchocarpic acid. Robustic acid is also definitely acidic in properties and can be titrated with alkali and it also forms a sparingly soluble potassium salt. Here too the acidic properties are due to a strongly acidic hydroxyl group which again does not give the ferric chloride colour. Like the other

two non-rotenoids it is also stable to treatment with aqueous alcoholic alkali. The potassium salt separates out and no reaction takes place. Robustic acid can be distinguished by the brilliant red colour which it finally gives in the Durham test.

The oxidation of robustic acid with alkaline hydrogen peroxide has now been carried out. *p*-Hydroxy benzoic acid could readily be separated from the products. By the action of bromine on robustic acid, its methyl ether and acetate a tetrabromo-compound is formed. This is a marked difference between this and the other two non-rotenoids.

Robustic acid again resembles scandenin and lonchocarpic acid in not giving chemical tests characteristic of a carbonyl group. Its ultra-violet absorption spectrum is almost identical with those of the other two non-rotenoids mentioned above. It also has some definite toxicity though it is also weak like the others.

It has already been mentioned that scandenin, lonchocarpic acid and robustic acid are extraordinarily stable to aqueous and alcoholic alkali. But their fully methylated ethers on the other hand, undergo decomposition easily with boiling aqueous alcoholic potash in the presence of zinc. The products consist mainly of neutral components isolated as viscous oils and the alkali-soluble portions are very small. There is correspondence in the behaviour of the three substances in this respect. The characterisation of the products is in progress.

Experimental

Scandenin

Molecular size.—As determined by the Micro-Zeisel method the methoxyl was 7·3%. From this the minimum molecular weight would be 425. Micro-titration using an alcoholic solution of scandenin and 0·1 N aqueous alkali also gave the value 425 for the equivalent weight (minimum molecular weight).

For obtaining the potassium salt, scandenin (1·0 g.) was dissolved in 2% potassium hydroxide (30 c.c.) by warming and the clear solution obtained after filtration was cooled. Large crystals of the potassium salt began to separate out and appeared as needles under the microscope. They were collected on a filter and washed with water to free them from adhering alkali. Finally they were washed with a small quantity of alcohol and dried in air.

The potassium salt (233·1 mg.) on ignition with two drops of concentrated sulphuric acid gave potassium sulphate (48·5 mg.). Therefore the minimum molecular weight of the potassium salt of scandenin is 418 or that

of scandenin 380. The higher yield of potassium sulphate and hence the lower value for the molecular weight of scandenin by this method may be due to some potassium hydroxide being retained by the potassium salt.

By Rast's method the molecular weight of scandenin was found to be 415, and that of its methyl ether 430. All the above results agree within limits of error with the molecular formula $C_{26}H_{26}O_6$ having a molecular weight of 434.

Sodium Salt.—Scandenin (40 mg.) was taken in chloroform (10 c.c.) and was extracted with 5% sodium carbonate solution (25 c.c.). In the first 5 minutes, turbidity developed with the separation of a solid at the interface. By shaking the mixture for some time and leaving overnight, a considerable amount of solid separated. It was filtered, washed successively with a small quantity of water, alcohol and ether and dried in air. The product left an ash on ignition, was soluble in warm water and the solution yielded scandenin on acidification. The sodium carbonate extract on acidification yielded some scandenin.

When the experiment was carried out with 5% sodium bicarbonate, neither was there any separation of the salt nor did the bicarbonate solution give any solid on acidification. Scandenin was therefore insoluble in bicarbonate.

Diacetate.—The preparation of the diacetate has already been described.[2] Its deacetylation could be effected as follows. The acetate (0·1 g.) was treated with 8% alcoholic potash (25 c.c.) when it readily went into solution giving a deep yellow colour. The solution was refluxed for ½ hour on a water-bath. Most of the alcohol was then distilled off after addition of sufficient amount of water (30 c.c.). No solid separated at this stage, thereby indicating complete hydrolysis of the acetate. The alkali solution was then acidified, the solid filtered and recrystallised from alcohol. It had a melting point 230–31° which was undepressed by admixture with scandenin.

Dimethyl ether.—The preparation of the dimethyl ether using dimethyl sulphate and anhydrous potassium carbonate in dry acetone medium was already described. The following procedure could also be adopted.

Scandenin (1·0 g.) was dissolved in acetone (50 c.c.) and to it was added alternately in small quantities 5% aqueous alkali and dimethyl sulphate (10 c.c.) keeping the solution slightly alkaline throughout. The addition of the methylating agent was carried out in the course of 2 hours. At this stage a crystalline solid began to separate. The solution was kept overnight and then the excess of dimethyl sulphate decomposed by heating with alkali,

After the addition of a large volume of water, the crystalline material (0·85 g.) that separated was filtered. It was purified by treatment with ether, when some ether-insoluble material separated out. This was found to be the potassium salt of scandenin. The ether-soluble portion consisted of a crystalline solid (0·75 g.) which on recrystallisation from alcohol melted at 129°. It agreed with the sample of methyl ether obtained by methylation under anhydrous conditions.

Oxidation of Scandenin with Hydrogen Peroxide.—During the war strong solutions of hydrogen peroxide were not available for this experiment. The dilute solution used for pharmaceutical purposes was found to be unsuitable. However, a sample of 40 volume hydrogen peroxide gave good results and the procedure adopted is given below.

Scandenin (1·0 g.) was dissolved in 2% alkali (60 c.c.) and to the hot solution was added 8 c.c. of 40 vol. hydrogen peroxide in small portions (0·5 c.c. at a time). Towards the last stages some more alkali was added to keep the substance in solution. After complete addition of the hydrogen peroxide, the solution was boiled, cooled and filtered. Carbon dioxide was passed into the clear alkali solution and it was ether-extracted to remove the bicarbonate insoluble portion. This consisted mostly of resinous material and a small quantity of unoxidised scandenin.

The bicarbonate solution was acidified with hydrochloric acid and repeatedly extracted with ether. The combined ether extract was dried over anhydrous sodium sulphate and evaporated off. The oily liquid that remained behind gradually solidified (clusters of needles). It was purified by dissolving it again in 5% sodium bicarbonate solution and by extracting the acidified solution with ether. Finally it was recrystallised from water using a little animal charcoal. It melted at 210–11°, gave a reddish brown colouration with ferric chloride and the mixed melting point with an authentic sample of *p*-hydroxy benzoic acid was undepressed.

Lonchocarpic Acid.—The sample of lonchocarpic acid was supplied to us by the Insecticides Division of U.S. Department of Agriculture, Washington, under instructions from Dr. H. A. Jones. It was from lot No. 28–51. According to the information sent along with it, it was obtained from the original crude crystalline product of the extraction of the plant material by washing with hexane and crystallising twice from ethyl alcohol. It melted at 215–16° with sintering at 202°. It was further purified by recrystallisation from alcohol and then it melted at 220–21° without any sintering earlier. Mixed melting points with scandenin and also 'nallanin'[2] were considerably depressed. It was found to be more easily soluble in alcohol

than scandenin, was readily soluble in 5% sodium carbonate solution from which it was precipitated by the addition of acids. It did not form a sparingly soluble alkali salt like scandenin. In the Durham test, the change was from greenish yellow to brick red. With concentrated sulphuric acid a deep red solution was obtained.

The acetate was prepared by heating the substance with acetic anhydride and sodium acetate for 2 hours. It crystallised from ethyl acetate petroleum-ether mixture as rectangular rods and melted at 153–4°. The methylation of lonchocarpic acid was effected by boiling it with excess dimethyl sulphate and anhydrous potassium carbonate in anhydrous acetone medium for 12 hours. The dimethyl ether crystallised from alcohol as rectangular prisms melting at 154–55°. These agreed with the description of Jones and Haller.[4]

Robustic Acid.—Most of the properties of robustic acid have already been described.[6] Its oxidation with alkaline hydrogen peroxide was done as follows:

Robustic acid (1·0 g.) was dissolved in 2% sodium hydroxide (50 c.c.) and to the hot solution was added hydrogen peroxide (100 vols.; 10 c.c.) in small portions. Other details of the experiment were the same as in the oxidation of scandenin described earlier in this paper. No unoxidised robustic acid was found in this case and *p*-hydroxy benzoic acid was readily isolated from the acid fraction; its identity was established by comparison with an authentic specimen.

Bromo-derivatives.—The bromo derivatives were prepared by adding a solution of bromine in glacial acetic acid to a solution of the substances in the same solvent until the yellow colour persisted. The products were thrown out of the solution by the addition of a large volume of water and purified by recrystallisation from acetic acid. They are colourless solids with the exception of scandenin bromide which is yellow and are microcrystalline powders; they are easily soluble in ethyl acetate and benzene, moderately soluble in alcohol and acetic acid and very sparingly soluble in petroleum ether. They do not melt and run down the capillary tube at their melting points but either decompose or become transparent glassy masses sticking to the sides of the tube. The bromo-derivatives obtained from robustic acid, its methyl ether and acetate give characteristic deep blue solutions in sulphuric acid.

The following table gives a summary of the data relating to the bromo-derivatives.

Compound	Melting point	Bromine % Found	Bromine % Calculated for
1 Scandenin bromide ..	125° d.	27·4	$C_{26}H_{26}O_6Br_2$, 26·7
2 Scandenin acetate bromide ..	119–20° d.	22·9	$C_{30}H_{30}O_8Br_2$, 23·5
3 Scandenin methylether bromide ..	109° d.	26·6	$C_{28}H_{30}O_6Br_2$, 25·6
4 Lonchocarpic acid acetate bromide ..	125° d.	23·2	$C_{30}H_{30}O_8Br_2$, 23·5
5 Lonchocarpic acid methyl ether bromide ..	113° d.	25·9	$C_{28}H_{30}O_6Br_2$, 25·6
6 Robustic acid bromide ..	144°	46·3	$C_{22}H_{20}O_6Br_4$, 45·7
7 Robustic acid acetate bromide ..	148°	43·8	$C_{24}H_{22}O_7Br_4$, 43·1
8 Robustic acid methyl ether bromide ..	112°	44·0	$C_{23}H_{22}O_6Br_4$, 44·8

A preliminary study of the ultra violet absorption spectra of scandenin, lonchocarpic acid and robustic acid in chloroform solution has now been made. The absorption regions at different concentrations agree closely and the maximum absorption for 0·002% solution is found between 3700 and 3100 A.U. Under the same conditions rotenone has the absorption band between 3300 and 2700 A.U. (see also Cahn, Phipers and Boam[5]). When compared with rotenone the absorption regions of the non-rotenoids are definitely displaced towards longer wave-lengths. The results would indicate the presence of a carbonyl group in conjugation with ethylenic bonds.

Substance	Absorption region
1 Rotenone ..	3300–2750 A.U.
2 Scandenin ..	3700–3100 ,,
3 Lonchocarpic acid ..	3700–3100 ,,
4 Robustic acid ..	3720–3000 ,,

Toxicity to fish.—Small fresh-water fish, *Haplochilus panchax*, were employed. The details of the method are the same as already described by Krishnaswamy and Seshadri.[7] The results are given in the following table:

ge = gelatin (1 gram) added per litre of water (see Murti and Seshadri).[8]

Compound	Concentration mg./litre	Turning time in minutes	Remarks
Scandenin ..	20	21·0	
	40	11·0	
Scandenin methyl ether ..	50	No effect in 4 hours	
Lonchocarpic acid ..	50	21·0	The fish did recover and died
Robustic acid ..	50	25·5	
Rotenone ..	1	6·5	

Our thanks are due to Dr. H. A. Jones for the sample of lonchocarpic acid used in this investigation.

SUMMARY

A more detailed study of scandenin is made and its important properties compared with those of lonchocarpic and robustic acids. The three non-rotenoids show marked resemblance. They do not give the Durham test, and are definitely acids owing this property not to carboxyl groups but to specially active hydroxyl groups. They are quite stable to aqueous and alcoholic alkali. The fully methylated ethers have three methoxy groups in all the three cases and these ethers undergo decomposition readily in alcoholic alkali in the presence of zinc, the main products being neutral in nature. The three compounds do not give chemical tests for the presence of carbonyl groups, exhibit definite toxicity to fish and have very similar ultra-violet absorption spectra. The toxicity and the spectra indicate the presence of carbonyl groups in conjugation with ethylenic bonds.

Oxidation with alkaline hydrogen peroxide yields *p*-hydroxy-benzoic acid with all the three compounds. In acetic acid solution scandenin and lonchocarpic acid add on two atoms of bromine; whereas robustic acid takes up 4 bromine atoms.

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ERRATUM

In Part II, Chemical Components of *Derris scandens*, *Proc. Ind. Acad. Sci.*, A, 1946, **24**, 374, *read* 'South Chanda Division' *for* 'South Canada Division'.

ANTHELMINTHICS—PART III

Anthelminthic Activity of *Calycopteris floribunda*

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The copper coloured tender leaves of *Calycopteris floribunda* Lamk. are reputed to have laxative and anthelminthic properties (Nadkarni)[1]: ground into paste and made into five grain pills they are administered for the expulsion of roundworms.

Ratnagiriswaran, Sehra and one of us[2] isolated from an acetone extract of the leaves a bright yellow crystalline substance, Calycopterin, and showed it to be a dihydroxy-tetramethoxyflavone, identified by Shah, Virkar and one of us[3] later as 5:4′-dihydroxy-3:6:7:8-tetramethoxyflavone. This constitution has been confirmed by its synthesis due to Seshadri and Venkateswarlu.[4]

On the strength of the statement of Nadkarni[1] and a report from Dr. K. Venkatachalam, Pharmacology Research Officer, Medical College, Madras, anthelminthic properties were first ascribed to calycopterin.[2] Mahal,[5] however, has since reported that calycopterin as well as other flavones examined by him had no effect on roundworms (*Ascaris lumbricoides*) and tapeworms (*Tænia serrata*). The present work was undertaken to determine if there was some constituent of *Calycopteris floribunda* leaves other than Calycopterin which was responsible for the reputed anthelminthic action of the drug.

For testing the various extracts of the leaves and comparing them with other anthelminthics, we have used earthworms, based upon the work of Sollman[6] who has shown that all anthelminthics are toxic to earthworms; a substance which is not toxic to these worms is scarcely worth further trial, while a positive result indicates that it has possibilities as an anthelminthic.

Following the experimental procedure described by Lamson and Brown,[7] except for the replacement of *Ascaris lumbricoides* by earthworms, we have found it preferable to note (*a*) the minimum time required to kill half the number of worms kept in a solution or suspension of the drug; (*b*) the time required to kill all the worms; and (*c*) minimum time required to cause fatal injury but not actual death.

In order to determine the best solvent for extracting the active constituent of the leaves, an infusion, a decoction, an alcohol extract and an acetone extract were prepared, and the anthelminthic activity of these extracts and

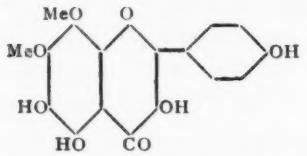
of the residue was estimated. The acetone extract proved to be the most effective, and as acetone had been the solvent used by all the previous workers for the isolation of calycopterin, calycopterin was prepared in the pure state and its activity compared with that of well-known anthelminthics. A few derivatives of calycopterin have also been examined, these being calycopteretin,[2] calycopterin dimethyl ether,[8] calycopterin-4′-monomethylether,[9] and calycopteretin-7 : 8-dimethylether.

By the action of aluminium chloride on calycopterin under specified conditions, two methyl groups are removed.[10] Calycopterin contains a hydroxyl group in the 5-position, and it has been shown that a 3-methoxyflavone undergoes demethylation with aluminium chloride.[3] Demethylcalycopterin exhibits the characteristic catechol colorations, and it is therefore constituted as calycopteretin 7 : 8-dimethyl ether (3 : 5 : 6 : 4′-tetrahydroxy-7 : 8-dimethoxyflavone).

Experimental

Calycopteretin 7 : 8-*dimethyl ether* (3 : 5 : 6 : 4′-*tetrahydroxy*-7 : 8-*dimethoxyflavone*)

Addition of calycopterin (2 g.) to a clear solution of aluminium chloride (2 g.) in nitrobenzene (10 c.c.) immediately produced a colour change to



deep orange. After heating the mixture on the water-bath for one hour, the viscous, deep orange product was treated with ice and hydrochloric acid, the nitrobenzene steamdistilled and the aqueous solution filtered hot. Bright yellow crystalline material (0·7 g.) separated overnight, recrystallisation from sulphurous acid yielded bright yellow needles, m.p. 268–272° (Found in material dried over phosphorous pentoxide in a desiccator, when the colour changed to a dull grey: C, 52·6; H, 4·4; $C_{17}H_{14}O_8$, 2 H_2O requires C, 53·4; H, 4·7%. Loss of weight on heating at 120° for 4 hours: 9·6%; $C_{17}H_{14}O_8$, $2H_2O$ requires H_2O, 9·4%). The hydroxyl content was determined by the acetic anhydride-pyridine method,[11, 12] although only 0·1 g. of the dried material was available for the purpose and minimal amounts of 0·5 g. are required for accurate results [Found: OH, 18·3; $C_{17}H_{10}O_4$ $(OH)_4$ requires OH, 19·7%]. The substance is coloured brownish orange by sulphuric acid and the pale orange solution exhibits a weak green fluorescence.

The aqueous solution reduces ammoniacal silver nitrate in the cold, as well as boiling Fehling's solution. Lead acetate gives an orange brown precipitate, the mixture rapidly darkening and changing in colour to olive green. The solution in dilute caustic soda is first a greenish yellow, then leaf green, orange and finally brown. Addition of a few drops of very dilute ammonia to the aqueous solution of demethylcalycopterin produces a series of colour changes as in the case of calycopterin, myricetin, gossypetin and robinetin; pale green, pale bluish green, deep turquoise blue, dark blue, dull olive green; the orange and magenta colour observed with calycopterin were absent. The pale brown solution in alcohol has a weak green fluorescence; with magnesium and hydrochloric acid the solution first turns yellow, then yellowish orange and finally deep reddish orange; the ferric chloride coloration is an intense olive green (with separation of a blue-green precipitate), which changes with ammonia to a dark brown. The characteristic catechol reaction with ammonium molybdate and acetic acid[12] was clearly observed; carrying out the test with 2 c.c. of a dilute aqueous solution of the substance to which was added 0·5 c.c. of glacial acetic acid and 1 c.c. of 14% ammonium molybdate solution it was found that while catechol and demethylcalycopterin gave a reddish brown coloration, a solution of the ketone (*vide infra*) obtained by alkaline hydrolysis of calycopterin showed no colour change. Calycopterin, resacetophenone, 2-hydroxy-4-methoxyacetophenone 2:6-dihydroxyacetophenone and 1-acetyl-2-naphthol gave a negative test.

Preparation of extracts

1. *The infusion* was prepared by pouring 800 c.c. of boiling water over 320 g. of dried, coarsely powdered leaves, and straining after 15 minutes.

2. *Decoction.*—The dried coarsely powdered leaves (400 g.) were boiled with water (1 *l.*) for 15 minutes and strained.

3. *Alcohol and acetone extract.*—The leaves (400 g.) were refluxed with 95% alcohol or acetone (1 *l.*) for 2 hours and filtered. The solvent was recovered from the filtrate, and the residue suspended in (*a*) distilled water, or (*b*) 1% sodium bicarbonate solution.

Toxicity of the extracts to earthworm

Six worms were placed in each of the solutions or suspensions mentioned above, and the time when all the worms were dead was noted. After the worms were dead, they were placed in fresh-water for 24 hours to make sure that they did not recover. The results are recorded in Table I. A blank experiment showed that the worms were alive in 1% sodium bicarbonate solution after 20 hours.

TABLE I

	Infusion	Decoction	Alcohol extract	Alcohol extract with 1% $NaHCO_3$
Time in minutes ..	365	340	225	215
	Acetone extract	Acetone extract with 1% $NaHCO_3$	Acetone extract hydrolysed by HCl	Acetone extract hydrolised by NaOH
Time in minutes ..	115	100	85	70

Comparison of Calycopterin with other anthelminthics

The drugs were dissolved in the minimum quantity of N/10 sodium hydroxide and made up to volume with tap water. Six worms were placed in each solution and determinations made as above. In two experiments,

TABLE II. *Determination of minimum time required to cause fatal injury*

Dilution	1: 10,000		1: 4,000		1: 2,000		1: 1,000		1: 200	
	A	B	A	B	A	B	A	B	A	B
Carbon tetrachloride ..	75	65	65	50	50	40	30	25	20	15
Carbon disulphide ..	85	75	80	70	65	60	55	45	30	25
Tetrachloroethylene ..	65	55	55	45	45	35	25	20	15	10
Thymol ..	80	70	70	60	60	50	50	40	35	25
β-Naphthol ..	45	35	35	30	30	20	20	15	10	8
Hexylresorcinol ..	35	30	30	25	20	15	12	10	8	6
Santonin ..	Over 4hours		190	170	165	145	130	115	90	75
Oil of chenopodium ..	210	195	155	130	120	100	85	70	50	40
Calycopterin ..	135	125	105	95	70	65	65	50	45	40
Calycopteretin-7: 8 dimethyl ether	155	140	115	100	100	90	85	75	60	55
Calycopteretin ..	175	160	150	135	125	120	100	90	80	70
Calycopterin-4′-monomethyl ether	Insoluble in NaOH : 6 worms lived for 24 hours in concentrated solutions (0·1% and 0·5%), and were found to be quite healthy when transferred to fresh tap water and observed after 24 hours.									
Calycopterin dimethyl ether										
Santonin + Bile salts ..	210	195	155	130	120	100	85	70	50	40
Calycopterin + BileSalts ..	280	270	240	230	220	210	170	160	140	130
Calycopteretin-7: 8-dimethyl-ether + Bile salts	300	285	255	240	230	215	175	160	150	140
Calycopteretin + Bile salts	315	305	275	260	245	235	195	180	180	170
Calycopterin − 4′-monomethyl ether + Bile salts	6 worms lived for 24 hours in concentrated solutions (0·1% to 0·5%) and were found to be quite healthy when transferred to fresh tap water and observed after 24 hours.									
Calycopterin and Gum acacia	305	295	265	255	235	225	200	190	160	145
Calycopteretin-7: 8-dimethyl ether + Gum acacia	325	315	270	260	250	235	210	190	175	160
Calycopteretin + Gum acacia	350	330	290	280	260	245	235	220	200	190

A: Time required to kill all the 6 worms. B : Time required to kill 3 worms.

however, instead of dissolving the calycopterin in sodium hydroxide solution it was dispersed in water with the aid of gum acacia and bile salts respectively. The results are given in Table II; the figures indicate the time in minutes required for killing (A) all the worms and (B) half of the worms placed in the given strength of solution.

The solid drugs mentioned below were dissolved in the minimum quantity of sodium hydroxide and made up to volume with tap water. Calycopterin, calycopteretin 7:8-dimethyl ether and calycopteretin were also separately suspended in tap water with the help of gum acacia or bile salts. A number of worms were placed in each solution or suspension and two at a time removed at definite intervals were washed and placed in fresh tap water. The figures D and C in Table III indicate the time in minutes when one or both the worms removed from the solution and placed in fresh tap water died within 24 hours.

TABLE III

Dilution	1: 10,000		1: 4,000		1: 2,000		1: 1,000		1: 200	
	C	D	C	D	C	D	C	D	C	D
Carbon tetrachloride ..	50	40	45	40	30	30	20	15	10	8
Carbon bisulphide ..	70	60	65	60	45	40	40	30	20	15
Tetrachloroethylene ..	45	40	40	35	30	25	15	10	8	8
Thymol ..	60	55	50	45	45	35	35	30	20	20
β-Naphthol ..	35	30	25	20	15	15	10	8	5	5
Hexylresorcinol ..	25	20	20	15	10	8	8	5	4	4
Santonin ..	180	160	150	140	135	130	70	70	65	60
Oil of chenopodium ..	180	170	110	100	90	85	55	45	35	30
Calycopterin ..	115	115	80	80	60	55	45	40	35	30
Calycopteretin 7:8-dimethyl ether	140	125	95	85	80	75	65	65	50	40
Calycopteretin ..	155	150	135	130	110	100	85	75	65	65
Santonin + Bile salts ..	125	110	80	75	60	60	55	45	25	20
Calycopterin + Bile salts ..	260	255	220	210	190	180	130	125	100	90
Calycopteretin 7:8-dimethyl-ether + Bile salts	265	250	220	200	180	180	135	125	105	95
Calycopteretin + Bile salts	300	290	240	230	205	295	170	165	150	140
calycopterin + Gum acacia	275	270	235	235	215	210	175	170	130	110
Calycopteretin-7:8-dimethyl ether + Gum acacia	280	275	235	230	220	215	170	165	140	125
Calycopteretin + Gum acacia	290	280	260	250	235	225	205	205	180	170

DISCUSSION

From the results obtained it is evident that calycopterin is toxic to earthworms, and other constituents of *Calycopteris floribunda* have no anthelminthic action. In studying the action of various drugs on the earthworms the time (*a*) required to kill all the worms kept in the solutions as well as the

time (*b*) required to kill only half the worms were recorded. This was to detect if some of the earthworms were unusually resistant to any of the drugs; but throughout the present experiments the times recorded for (*a*) and (*b*) were very close to each other.

When the minimum time required to cause fatal injury to the worms by a given dilution of the drug is compared with the time required to actually kill the worms still in contact with the same dilution of the drugs, some interesting results are observed. Thus a 1 : 10,000 solution of carbon tetrachloride requires 75 minutes of contact to kill all the worms, but only 50 minutes of contact are required to cause fatal injury while for carbon disulphide the time periods are 85 and 70 minutes. Considerable variations are noticeable in other cases also.

Comparing the toxicity of calycopterin to earthworms with other anthelminthics it is clear that, while it is not so toxic as carbon tetrachloride, carbon bisulphide, hexylresorcinol, etc., it is more toxic than the natural anthelminthics such as santonin and chenopodium. The greater toxicity of calycopterin in comparison with santonin and oil of chenopodium becomes much more marked at higher dilutions. At 1 : 10,000 dilution worms keep living in santonin for more than 4 hours, in oil of chenopodium they die in 210 minutes, while in calycopterin solution they die in 135 minutes.

In view of the indication of some anthelminthic action in calycopterin a more detailed examination of a series of flavones and flavonols has been projected. Considering the derivatives of calycopterin which we have now studied, it is interesting to note that, when all the hydroxyl groups are free, the compound (calycopteretin) is less toxic than calycopterin and toxicity increases as the number of methoxy group increases till calycopterin is reached. Proceeding further, calycopterin 4′-monomethyl ether and calycopterin dimethyl ether are both insoluble in alkali and hence less toxic than calycopterin. Seshadri and Viswanandham[9] have studied the toxicity of a number of flavones as fish poisons. Toxicity towards fish is obviously not parallel to toxicity towards earthworms (Paranjape, Phalnikar, Bhide and Nargund)[13] but among the flavones studied by Seshadri and Viswanadham it was observed that if all the hydroxyl groups of a flavone are free, the compound is relatively less toxic than the partially *o*-alkylated derivatives. On the other hand, one or more free hydroxyl groups are always required to impart the necessary solubility. As reported in this paper, aqueous dispersions of insoluble compounds using gum acacia or bile salts are not as effective as true solutions. In this connection and from other points of

view, we are examining the effect of surface-active compounds on anthelminthic action.

Summary

Various extracts of the leaves of *Calycopteris floribunda* have been prepared, and their toxicity to earthworms studied under standard conditions, in comparison with well-known anthelminthics. Calycopterin has been found to be more toxic to earthworms than santonin or oil of chenopodium, but less toxic than carbon tetrachloride, thymol, etc. Demethylation as well as methylation of calycopterin diminishes anthelminthic activity.

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INSECTICIDAL PROPERTIES AND CHEMICAL CONSTITUTION

Part V. Flavanones and Chalkones

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Since flavanones have a dihydropyrone ring they resemble in structure rotenone and its allies more closely than the flavones do. Consequently a study of their toxic properties will be useful. They occur fairly widely in nature mainly in the form of glycosides. Except for the capacity of hesperidin and eriodictyn to serve as vitamin-P[1] and the marked bitterness of naringin,[2] very little is known about their physiological properties. The chalkones are closely related to flavanones and also occur in nature though not so widely. The reputed anthelmintic property of kamala seems to be due to the presence of rottlerin[3] which has the chalkone structure.[4] This indicated the need for the study of the simpler chalkones also.

The method adopted for the tests using fresh-water fish (*Haplochilus panchax*) has already been indicated in our earlier publication on this subject.[5] It is desirable to use porcelain troughs for the experiments in order to minimise injury to the fish when they strike against the side in their anxiety to get away. It is also necessary to provide a glass cover for the trough since in most cases the fish attempt to jump out. The reactions of fish vary considerably under the influence of different substances. Some times an extraordinary tendency is exhibited to jump out; this is totally absent in some other cases; with some substances wriggling is prominent.

The following table gives a summary of the results using a number of flavanones and chalkones.

The chalkones and flavanones listed in the above table have been prepared following the methods described in the literature with slight modifications. The completely methylated chalkones have been obtained by the methylation of the corresponding 2-hydroxy compounds with dimethyl sulphate and potassium carbonate in anhydrous acetone medium. Derivatives of naringenin have been prepared by the partial and complete methylation of naringenin and of naringin accompanied by hydrolysis. Details will be given later in some other connection. Pedicellin required for the tests has been isolated from the leaves of *Didymocarpus pedicellata*.[13]

TABLE

(ge = gelatin added)

No.	Name of the compound	Concentration in milligrams per litre	Turning time in minutes	Remarks
1	7-Hydroxy-flavanone[6] ..	50 (ge)	16·0	Fish not recovered
		30 (ge)	27·0	
2	7-Methoxy-flavanone[7] ..	30 (ge)	4·0	
		20	6·0	
		10	12·0	
3	5-Methoxy-flavanone ..	10 (ge)	..	No effect in 2 hours
		20 (ge)	..	Turbidity developed
4	5-Hydroxy-7-methoxy-flavanone[8] ..	10 (ge)	20·5	
		20 (ge)	..	Solid separated
5	5 : 7-Dimethoxy-flavanone[7] ..	30 (ge)	3·5	
		20 (ge)	6·0	
		10 (ge)	16·0	
6	7-Hydroxy-4′-methoxy-flavanone[7] ..	40 (ge)	22·5	
		20 (ge)	120·0	
7	Naringenin (5 : 7 : 4′-trihydroxy-flavanone) ..	150	53·0	
		100	90·0	
8	Isosakuranetin(4′-methyl-ether of Naringenin)	40 (ge)	31·5	
		20 (ge)	285·0	
9	Naringenin-7 : 4′-dimethyl-ether ..	10 (ge)	17·0	Fish exhibit marked tendency to jump
		20 (ge)	..	Turbidity developed
10	5 : 7 : 4′-Trimethoxy-flavanone (Naringenin-trimethyl-ether)	20 (ge)	9·0	
		10 (ge)	29·5	
11	4′-Methylether of butin[9] ..	50 (ge)	..	No effect in 20 hours
12	Butin trimethylether[10] (7 : 3 : 4′trimethoxy-flavanone)	20	9·0	
		10	21·0	
13	5 : 7 : 3′: 4′-tetramethoxy-flavanone[11] ..	40 (ge)	16·5	
		20	40·0	
14	2 : 4-Dihydroxy-chalkone[6] ..	60 (ge)	14·0	
		40 (ge)	19·5	
15	2-Hydroxy-4-methoxy-chalkone[12] ..	10 (ge)	..	Fish slightly affected and blackening was noticed. Solid separated after 2 hours
16	2-Hydroxy-6-methoxy-chalkone ..	20 (ge)	7·5	Recovery slow
		10 (ge)	17·0	
17	2 : 4-Dihydroxy-4′-methoxy chalkone[7] ..	30 (ge)	9·0	
		20 (ge)	15·0	
18	2-Hydroxy-4: 4′-dimethoxy-chalkone ..	20 (ge)	..	Separation of solid
		10 (ge)	..	do
19	2-Hydroxy-4 :6-dimethoxy-chalkone[7] ..	30 (ge)	12·0	
		20 (ge)	18·0	
20	2-Hydroxy-4 : 6 : 4′-trimethoxy-chalkone ..	10 (ge)	..	Solid separated. Slight toxicity. Fish exhibit tendency to jump
21	2 : 4 : 4′-Trihydroxy-3′-methoxy-chalkone ..	50	24·5	Fish blackened
		40	29·0	
22	2-Hydroxy-4 : 3′ : 4′-trimethoxy-chalkone[10] ..	10 (ge)	59·0	
		20 (ge)	..	Solid separated
23	2 : 4 : 6-Trimethoxy-chalkone ..	20 (ge)	5·0	Violent jumping
		10 (ge)	12·5	
24	2 : 4 : 6 : 4′-Tetramethoxy-chalkone ..	30 (ge)	9·5	Violent jumping
		20 (ge)	14·0	
25	Pedicellin[13] ..	30 (ge)	9·0	Slight reddening
		20 (ge)	18·0	

With regard to their toxicity, the flavanones resemble the flavones in a general way. They are definitely and markedly toxic thus supporting the presence of a toxophore in them. The simpler hydroxy and methoxy compounds are highly toxic and the toxicity falls off with increase in these groups (compare 2, 5, 10, 12 and 13). The methyl ethers are far more toxic than the corresponding hydroxy compounds. Further the presence of more than one methoxyl in the side-phenyl nucleus brings down the potency markedly (compare 10 and 13) and in some compounds the presence of a free 5-hydroxyl enhances toxicity (compare 9 and 10) but not in all cases. There is a big drop in toxicity from isosakuranetin (8) to naringenin (7); this may indicate the marked effect of a free hydroxyl in the 4′-position. Compared with the corresponding derivatives of flavones, the methyl ethers of flavanones are somewhat less toxic, whereas the hydroxy compounds are more toxic. For example, 7-methoxy-flavanone is less toxic than 7-methoxy-flavone which gives a turning time of 5 minutes for 10 mg. It approximates to the methoxy-phenyl-coumarins. On the other hand, 7-hydroxy-flavanone is considerably more toxic than 7-hydroxy-flavone and it approximates to 3-phenyl-umbelliferone. It is remarkable that 5-methoxy-flavanone has no marked toxic property whereas the isomeric 7-methoxy-compound is so powerful. Similar lowering of toxicity has already been noted in regard to 5-methoxy-flavone also (see Part IV[14]).

The chalkones are also definitely toxic and the effect of adding hydroxyl and methoxyl groups are in general the same as in the flavanones. In the case of some of the hydroxy compounds though the toxic effect was slow in coming on, the recovery of the fish when returned to fresh water was also slow and in some cases they died. Such an effect in a much more marked manner has been noticed with rottlerin[15] which is also a hydroxy-chalkone; in this case the fish do not recover at all and die immediately after turning upside down. Comparing the flavanones with the corresponding isomeric chalkones the following points emerge. Hydroxy-flavanones (1, 6 and 11) are much weaker than the isomeric chalkones (14, 17 and 21) whereas with the flavanone methyl ethers the reverse is the case (compare 2, 5, 10 and 12 with 15, 19, 20 and 22). It should be noted however as an exception that 2-hydroxy-6-methoxy chalkone (16) is markedly toxic whereas the isomeric 5-methoxy flavanone (3) has very little toxicity. A further interesting feature is that as soon as the remaining ortho-hydroxyl group in the chalkone is also methylated the fully methylated chalkones are considerably more toxic (see 23 and 24). In this connection the behaviour of the naturally occurring chalkone pedicellin (25) is interesting. Though it has five methoxyl groups it is appreciably toxic to fish.

Summary

The toxic properties of a number of simple hydroxy and methoxy flavanones and chalkones have been studied using fresh-water fish. The flavanones resemble the flavones in a general way. However the methyl ethers of the flavanones appear to be less toxic than the corresponding flavone derivatives, whereas with the hydroxy compounds the reverse seems to be the case.

With the chalkones the toxic symptoms set in more slowly but they are more persistent. The methoxy chalkones are less toxic than the isomeric flavanones whereas when a number of hydroxyl groups are present the reverse is the case. As soon as all the hydroxyl groups in chalkones are methylated the toxicity increases considerably.

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ULTRASONIC STUDIES IN AMETHYST AND SMOKY QUARTZ

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1. Introduction

Investigations relating to the elastic behaviour of the naturally coloured quartz like the Amethyst and Smoky varieties have been very scanty. Extensive and precise work had however been done on colourless quartz by Voigt[1] and others. Most of the work done on the coloured varieties pertained to the study of the occurrence and genesis, effect of heat on their colours, the influence of radiations, the transmission of light through them, etc. Such studies were mainly intended to explore the possible source of their colouration. To investigate how far the colour and other structural imperfections like the twinning, often associated with colour, influence their elastic behaviour, and with a hope that such work may shed light on the possible cause of colour, the author has undertaken the ultrasonic studies of coloured varieties. The work in Amethyst deals with the study of transmission frequencies on ten 'Z' sections, and piezoelectric frequencies on four minor rhomb sections of different shades of colour. They have also been studied after decolourisation by prolonged heating. Four Smoky quartz sections, of random orientations, with different shades of colour, are also studied by utilising their piezoelectric frequencies. The heat treatment for these specimens is repeated in a slightly new and different way.

2. Experimental Methods

The study of transmission frequencies has been done by the Wedge method, evolved in this laboratory, and described in an earlier paper by Bhagavantam and Bhimasenachar.[2]

The phenomenon of diffraction of light by ultrasonic waves discovered by Debye and Sears,[3] is employed for the detection of resonant frequencies. The electrical oscillator, used to excite the crystal is of the shunt-fed Hartley type, using a valve Mullard D.O. 24, and giving a frequency range from 2 to 18 megacycles per second. The resonant frequencies are measured

by a Phillip's Heterodyne Wavemeter. The densities of the specimens, before and after decolourisation, are determined by using a sensitive balance in the usual manner. The heat treatment and the decolourisation are effected by long continued heating in an air oven, and suitable arrangements to observe colour changes during heating have been made.

3. Results

Arbitrary intensity numbers, ranging from 100 for the most intensely coloured to 10 for the least coloured have been assigned to get an idea of the depth and general gradation of colour from specimen to specimen. In the 'Z' sections, both longitudinal and torsional modes have been recorded. Results are given in the following tables.

Table I

Amethyst—Transmission Frequencies

Serial No.	Intensity No.	Thickness m.m.	Coloured			Decolourised		
			Density	Long Fndl. Mc.	Torsion Fndl. Mc.	Density	Long. Fndl. Mc.	Torsion Fndl. Mc.
1	100	2·120	2·696	1·680	1·242	2·695	1·683	1·248
2	85	1·830	2·750	1·749	1·228	2·750	1·750	1·226
3	70	1·640	2·580	1·950	1·373	2·590	1·970	1·370
4	60	1·740	2·664	1·900	1·200	2·667	1·903	1·201
5	45	1·750	2·840	1·864	1·220	2·800	1·860	1·220
6	35	1·600	2·896	2·100	1·420	2·892	2·120	1·410
7	25	1·730	2·732	2·000	1·368	2·741	2·020	1·366
8	20	1·970	2·758	1·700	1·100	2·760	1·702	1·100
9	15	1·820	2·747	1·759	1·250	2·746	1·790	1·220
10	10	2·150	2·750	1·461	1·100	2·720	1·461	1·102

Table II

Amethyst-Piezoelectric Frequencies

Serial No.	Intensity No.	Thickness mm.	Coloured		Decolourised	
			Density	Fundamental Mc.	Density	Fundamental Mc.
1	70	1·560	2·812	1·143	2·810	1·140
2	60	1·690	2·717	1·123	2·718	1·124
3	30	1·697	2·882	1·062	2·880	1·062
4	20	1·700	2·810	1·090	2·810	1·085

TABLE III

Smoky Quartz—Piezoelectric Frequencies

Serial No.	Intensity No.	Thickness mm.	Coloured			Decolourised		
			Density	First Fndl. Mc.	Second Fndl. Mc.	Density	First Fndl. Mc.	Second Fndl. Mc.
1	80	1·504	2·680	1·881	1·310	2·690	1·881	1·310
2	60	1·504	2·690	1·940	1·543	2·670	1·945	1·540
3	30	1·507	2·670	1·889	1·256	2·670	1·904	1·254
4	20	1·506	2·700	..	1·343	2·730	..	1·360

4. CONCLUSIONS

An interesting feature is observed when we look at the longitudinal and torsional modes in 'Z' sections in Table I. The former is higher in all cases, and the latter is lower in many, than the corresponding values for colourless quartz. Even in the piezoelectric oscillations (Table II), shear frequencies with which the minor rhomb sections oscillate, are definitely less than that of colourless quartz. Further it has been found that a 'Y' cut plate, and major rhomb, both of which, like many others, being cut from thickly coloured portions, did not oscillate piezoelectrically. This is an interesting result. Such sections have been studied by the transmission method. The shear elastic constant C_{66} in a 'Y' cut section came out as $3{\cdot}16 \times 10^{11}$ which is definitely less than $3{\cdot}9\,(9) \times 10^{11}$ which is the corresponding value for colourless quartz. The shear frequencies of the major rhomb are also found to be definitely less than those of colourless quartz, thus confirming the observation already made.

The elastic constants C_{33} and C_{44} of which the longitudinal and the torsional fundamentals are a measure, and the piezoelectric frequencies of minor rhombs, vary very erratically, showing no correlative variations with the depth of colour.

The frequency of a finished Amethyst section is found to change from place to place, as indicated by the impaired resonance position in the wavemeter; and this is persistently observed in most of the sections worked with.

The densities of Amethyst, though slightly higher than that of colourless quartz, show no regular variation with the depth of colour.

Contrary to Amethyst, Smoky quartz sections oscillate quite powerfully, even without silvering, and the appearance of even overtones indicates the

presence of twinning. Random orientations of the specimens chosen render the conclusions regarding the variation of the elastic frequency with colour, uncertain. Unlike in Amethyst, the frequency throughout a finished Smoky quartz section is found to be uniform. The densities show no regular variation with colour.

5. Heat Treatment

A few sections of Amethyst and Smoky quartz have been heated, for 1½ hours in the case of Amethyst, and 1 hour in the case of Smoky quartz, at various temperatures, and at the end of this period the then existing colour is noted, by assigning intensity numbers. The colour begins to disappear markedly at a temperature between 260° C. and 275° C. in Amethyst and at about 230° C. in Smoky quartz.

By noting the time taken for complete decolourisation at 400° C. for various specimens, it is found that originally dark coloured specimens of Amethyst require a larger time for complete decolourisation at a particular temperature. The decolourisation of Smoky quartz is a time-temperature reaction, and it loses its colour completely even within 45 minutes at 400° C.

After complete decolourisation, the frequencies have been redetermined in all the sections studied. It is very interesting to note that both in Amethyst and Smoky, neither the elastic frequencies (transmission or piezoelectric) nor the densities are altered. The small changes that may be seen in the tables are however well within the experimental limitations.

From Narayana Rao's[4] recent studies in dielectric properties, it is interesting to note that the elastic and the dielectric behaviours of Amethyst and Smoky quartz with colour and after decolourisation are closely analogous.

6. Discussion of Results

Holden[5] has suggested that the colour in Amethyst is caused by a metallic impurity, namely iron oxide. Though a slight rise in the elastic constant may be taken to signify the presence of a metallic impurity, Holden's suggestion, in the present case, is untenable, because variations in the elastic constants with respect to colour are quite erratic and show no correlation.

The suggestion that the colour is an inorganic dye, whose physical state of aggregation changes on heating, is also disputable, because even after complete decolourisation the elastic frequencies are unaltered. This excludes the possibility of the change of state of aggregation of the dye. If such a thing happened, it would have had a perceptible effect on the elastic properties.

The inconsistent variation of the elastic frequencies of Amethyst and Smoky quartz with respect to colour, also leads us to believe that probably they are less influenced by the colouring agent, whatever it be, than by the structural imperfections like the excessive twinning associated with them. Lukesh's[6] work on imperfect quartz, where twinning is found to have a deleterious effect on its elasticity, supports this author's above suggestion. The non-uniformity in frequency in a finished single Amethyst section, can therefore be looked upon as due to the presence of different amounts of twinning in different regions.

The fact that continued effort to oscillate piezoelectrically 'Y' cut plates, cut from portions of very dense colour, met with failure, suggests that probably the excessive twinning of Amethyst may have something to do with colour, though the exact nature of relation cannot be understood at this stage. The cause of colour of natural Smoky quartz seems to be different from that of Amethyst. Frondel,[7] however, made some observations on artificial Smoky quartz, " that twinning exerts a control over the distribution of colour " and that " the tendency to twin is related to the tendency of quartz to become smoky in colour ".

7. Summary

The piezoelectric frequencies of Amethyst and Smoky quartz, the transmission frequencies in Amethyst, and the densities of both, vary irregularly and show no correlative variation with depth of colour. In Amethyst the longitudinal frequencies of 'Z' sections are higher and shear frequencies in the 'Z', 'Y', minor rhomb and major rhomb, are lower than the corresponding values in colourless quartz. The frequency of a finished Amethyst section is not uniform throughout, whereas in Smoky quartz it is so. After complete decolourisation by heating, neither the elastic frequencies, nor the densities of Amethyst and Smoky quartz change. The elastic and dielectric behaviours of Amethyst and Smoky quartz, with colour and after decolourisation, are analogous in many respects.

The Amethyst begins to lose its colour markedly at a temperature between 260° C. and 275° C., and the Smoky quartz at about 230° C. Originally dark coloured specimens require longer times for decolourisation at a particular temperature.

In the light of the elastic data, the suggestion that metallic impurities cause the colour, is untenable. It is quite probable that the excessive twinning present in Amethyst has something to do with colour, though the exact

nature of relation cannot be set out at this stage. The cause of colour of natural Smoky quartz seems to be different from that of Amethyst.

The author in conclusion wishes to express his grateful thanks to Prof. S. Bhagavantam for his constant direction and interest.

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THE LANTHANUM NITRATE TEST FOR ACETATE IN INORGANIC QUALITATIVE ANALYSIS

Part II. Limits of Identification

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In a previous communication which may be regarded as Part I, Neelakantam and Row[1] introduced the lanthanum nitrate test for acetate into systematic inorganic qualitative analysis. This test which is the best available for the detection of acetate has been in regular use in our laboratories and proved to be of immense value in routine work. It was occasionally found, however, that the test failed to detect acetate. Krüger and Tschirch[2] noted that a large excess of acetate itself tended to transform the blue colour normally obtained in the test into an yellowish brown but in our experiments in some cases there was no development of colour at all. Our experience of the test indicated that this difficulty was primarily due to a high acetate ion concentration—an upper limit of identification existed, and to some extent on the amount of ammonia added. The addition of a drop or two of ammonia after the customary "nearly but not completely discharged colour of iodine" stage frequently secured a positive result in those cases wherein a negative one was reported according to the usual procedure. The lower limits of identification had previously been reported upon by other investigators but the upper limit which is of considerable importance in routine qualitative analysis has not been determined. The present paper deals with this aspect.

Experimental

Solutions

Acetic Acid.—Solutions of acetic acid of known strength ranging from 8 N down to 0·005 N were prepared.

Lanthanum Nitrate.—A 5% aqueous solution was prepared using a B.D.H. sample.

Iodine.—A 0·1 N solution of iodine in potassium iodide was used.

Procedure.—Acetic acid solution (1.0 c.c.) was pipetted into a test-tube and treated with the lanthanum nitrate solution (1·0 c.c.). Enough drops

of iodine solution were added to impart a distinct yellow colour to the solution and then dilute ammonia (2 N) was added dropwise until the yellow colour was nearly but not completely discharged. If no colour developed in the cold within a few minutes, the test-tube was placed in a boiling water-bath.

Results

Acetic acid (mg. per c.c.)	Colour	Remarks
480	Nil	None in the cold or on heating; none even on adding more ammonia.
240	Nil	None in the cold or on heating but on adding more ammonia a light purple tinge developed slowly in the cold but this was intensified very slightly on heating
180	Nil	do.
120	Light purple → blue	A light purple colour was immediately obtained; this turned purple within a minute and blue in about five minutes in the cold. Turbidity developed slowly in the cold and quickly in the hot
60·0	Deep purple → dark blue	A deep purple colour was immediately obtained and it turned dark blue rapidly in the cold. Turbidity developed slowly in the cold and quickly in the hot
30·0	do.	do.
12·0	Dark blue	A pure dark blue colour was immediately obtained in the cold. No turbidity in the cold.
6·0	Brownish purple → deep blue	At first a brownish purple which changed to pure deep blue rapidly in the cold. No turbidity in the cold.
3·0	Deep blue	Rapidly in the cold. No turbidity.
2·0	do.	do.
1·5	do.	Colour developed rapidly in the cold but the solution became turbid.
1·2	do.	do.
1·0	Blue	Developed slowly in the cold. Solution became turbid.
0·87	Greenish blue	do.
0·75	Dirty grey → deep blue	Gelatinous precipitate, light dirty grey in colour in the cold: on heating, deep blue
0·67	do.	do.
0·60	Nil → purple	Colour developed slowly on heating.
0·55	do.	do.
0·50	do.	do.
0·46	do.	do.
0·43	do.	do.
0·40	do.	do.
0·38	do.	do.
0·35	do.	do.
0·33	Purplish blue	do.
0·32	Bluish	do.
0·30	Light dirty blue	do.
0·24	Bluish grey	do.
0·20	Light bluish grey	do.
0·17	Nil	Cream coloured precipitate but no trace of purple or blue in the cold or on heating

Upper limit of identification 120 mg. per c.c.

Lower limit of identification 0·2 mg. per c.c.

The results obtained show that there is an upper limit of identification. A definite blue colour is obtained with a maximum of 120 mg. per c.c. of acetic acid. The lower limit is 0·2 mg. per c.c. or 1 in 5,000 (Feigl,[3] gave 1 in 2,000 for the micro-chemical test).

Summary

1. An upper limit of identification exists for the lanthanum nitrate test for acetate.

2. A definite blue colour is obtained in the test for concentrations of acetic acid ranging from 120 mg. to 0·2 mg. per c.c.

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ON THE RESULTANT OF TWO FUNCTIONS

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§1. Let us define the resultant of two functions $f_1(x)$ and $f_2(x)$ by the equation,

$$g(x) = \int_0^\infty f_1(y) f_2(xy)\, dy.$$

It is well known that if $f_1(x)$ and $f_2(x)$ are two Fourier kernels then $g(x)$ is also a Fourier kernel.[1] In case we consider kernels transforming one self-reciprocal function to another, it is possible to obtain a kernel $g(x)$ defined in the same manner as above, by choosing two suitable functions $f_1(x)$ and $f_2(x)$.

The main result of this paper is embodied in the following theorem:—

The resultant,

$$g(x) = \int_0^\infty f_1(xy) f_2(y)\, dy,$$

of two functions,[2]

$$f_1(x) = \frac{1}{2\pi i} \int_{c-i\infty}^{c+i\infty} 2^{s/2}\, \Gamma\left(\frac{1}{4}+\frac{\mu}{2}+\frac{s}{2}\right) \Gamma\left(\frac{1}{4}+\frac{\lambda}{2}+\frac{s}{2}\right) \times \Gamma\left(\frac{3}{4}+\frac{\nu}{2}-\frac{s}{2}\right) \omega(s)\, x^{-s}\, ds \qquad (1.1)$$

and

$$f_2(x) = \frac{1}{2\pi i} \int_{c'-i\infty}^{c'+i\infty} 2^{s/2}\, \Gamma\left(\frac{1}{4}+\frac{\mu}{2}+\frac{s}{2}\right) \Gamma\left(\frac{1}{4}+\frac{\theta}{2}+\frac{s}{2}\right) \times \Gamma\left(\frac{3}{4}+\frac{\nu}{2}-\frac{s}{2}\right) \omega'(s)\, x^{-s}\, ds \qquad (1.2)$$

where,

$$0 < c < 1, \qquad 0 < c' < 1$$

$$\omega(s) = \omega(1-s), \qquad \omega'(s) = \omega'(1-s)$$

is a kernel transforming R_λ* *into* R_θ.

* By a R_ν function we will mean a function self-reciprocal in the Hankel Transform of order ν.

Proof.—Consider the resultant function,

$$g(x) = \int_0^\infty f_1(xy)\, f_2(y)\, dy$$

$$= \frac{1}{2\pi i} \int_0^\infty f_2(y)\, dy \int_{c-i\infty}^{c+i\infty} 2^{s/2}\, \Gamma\left(\frac{1}{4}+\frac{\mu}{2}+\frac{s}{2}\right) \Gamma\left(\frac{1}{4}+\frac{\lambda}{2}+\frac{s}{2}\right)$$

$$\times\, \Gamma\left(\frac{3}{4}+\frac{\nu}{2}-\frac{s}{2}\right) \omega(s)\, x^{-s}\, y^{-s}\, ds$$

from (1.1).

$$= \frac{1}{2\pi i} \int_{c-i\infty}^{c+i\infty} 2^{s/2}\, \Gamma\left(\frac{1}{4}+\frac{\mu}{2}+\frac{s}{2}\right) \Gamma\left(\frac{1}{4}+\frac{\lambda}{2}+\frac{s}{2}\right)$$

$$\times\, \Gamma\left(\frac{3}{4}+\frac{\nu}{2}-\frac{s}{2}\right) \omega(s)\; x^{-s}\; ds \int_0^\infty f_2(y)\, y^{-s}\, dy$$

Now, applying Mellin's Inversion Formula to (1.2) and changing s to $(1-s)$ and also remembering that

$$\omega'(s) = \omega'(1-s),$$

we get,

$$\int_0^\infty f_2(y)\, y^{-s}\, dy = 2^{\frac{1-s}{2}}\, \Gamma\left(\frac{3}{4}+\frac{\mu}{2}-\frac{s}{2}\right) \Gamma\left(\frac{1}{4}+\frac{\nu}{2}+\frac{s}{2}\right)$$

$$\times\, \Gamma\left(\frac{3}{4}+\frac{\theta}{2}-\frac{s}{2}\right) \omega'(s)$$

Hence,

$$g(x) = \frac{1}{2\pi i} \int_{c-i\infty}^{c+i\infty} \Gamma\left(\frac{1}{4}+\frac{\lambda}{2}+\frac{s}{2}\right) \Gamma\left(\frac{3}{4}+\frac{\theta}{2}-\frac{s}{2}\right) \chi(s)\, x^{-s}\, ds$$

where,

$$\chi(s) = 2^{\frac{1}{2}}\, \Gamma\left(\frac{1}{4}+\frac{\mu}{2}+\frac{s}{2}\right) \Gamma\left(\frac{3}{4}+\frac{\mu}{2}-\frac{s}{2}\right) \Gamma\left(\frac{1}{4}+\frac{\nu}{2}+\frac{s}{2}\right)$$

$$\times\, \Gamma\left(\frac{3}{4}+\frac{\nu}{2}-\frac{s}{2}\right) \omega(s)\, \omega'(s)$$

Since,

$$\chi(s) = \chi(1-s)$$

$g(x)$ is a kernel[3] transforming $\mathbf{R}_\lambda$ to $\mathbf{R}_\theta$.

To justify the change in the order of integration we consider the integral,

$$\int_0^\infty |f_2(y)|\, dy \int_{c-i\infty}^{c+i\infty} \Big| 2^{s/2}\, \Gamma\left(\frac{1}{4}+\frac{\mu}{2}+\frac{s}{2}\right) \Gamma\left(\frac{1}{4}+\frac{\lambda}{2}+\frac{s}{2}\right)$$

$$\times\, \Gamma\left(\frac{3}{4}+\frac{\nu}{2}-\frac{s}{2}\right) \omega(s)\, x^{-s}\, y^{-s} \Big|\, ds \qquad (1.3)$$

Putting $s = c + it$ and using the asymptotic formula

$$\left| \Gamma\left(\mathrm{A} + i\frac{t}{2}\cdot\right) \right| \sim ce^{-\frac{\pi}{4}|t|} |t|^{\mathrm{A}-\frac{1}{2}} \text{ as } |t| \to \infty$$

we see that (1.3) is less than a constant multiple of

$$x^{-c} \int_0^\infty |f_2(y)|\, y^{-c}\, dy \int_{-\infty}^{\infty} |t|^{\frac{1}{2}(\mu+\nu+\lambda)+\frac{c}{2}-\frac{1}{4}} e^{-\frac{3\pi}{4}|t|} |\omega(s)|\, dt$$

The change in the order of integration will thus be permitted if both the y and the t-integrals exist.

They will exist[4] if both the functions $f_1(x)$ and $f_2(x)$ belong to A $(\alpha, a.)$*

Now, the modulus of the integrand of (1.1) is less than a constant multiple of

$$r^{-\sigma} |t|^{\frac{1}{2}[\mu+\nu+\lambda]+\frac{\sigma}{2}-\frac{1}{4}} e^{-|t|(\alpha-\eta-|\theta|)},$$

for,

$$\omega(s) = \mathrm{O}\left(e^{\left[\frac{3\pi}{4}-\alpha+\eta\right]|t|}\right)$$

for every positive $\eta < \alpha$, and uniformly in any strip interior to, $a < \sigma < 1 - a$, where c is any value of σ in the above strip.

Thus, the integral converges uniformly in any domain of x interior to the angle α and in any smaller angle is $\mathrm{O}(|x|^{-c})$. Since, c can be taken as near to $(1 - a)$ or a, as we please, $f_1(x)$ belongs to A (α, a). Similarly, $f_2(x)$ also belongs to A (α, a).

This completes the proof of the theorem.

To illustrate the above theorem, let us put,

$$\omega(s) = \frac{2^{\frac{\lambda}{2}-\frac{3}{4}}}{\Gamma\left(\frac{\nu}{2}-\frac{\mu}{2}+1\right)} \cdot \frac{1}{\Gamma\left(\frac{1}{4}+\frac{\mu}{2}+\frac{s}{2}\right)\Gamma\left(\frac{3}{4}+\frac{\mu}{2}-\frac{s}{2}\right)}$$

(1.1) gives the function,[5]

$$f_1(x) = x^{\lambda+\frac{1}{2}} e^{-\frac{1}{2}x^2} \mathrm{L}^{\frac{\mu+\lambda}{2}}_{\frac{\nu-\mu}{2}}(\tfrac{1}{2}x^2) \qquad (1.4)$$

where $\lambda \geqslant -\frac{1}{2}$, $\nu > -\frac{1}{2}$ and $(\nu - \mu)$ is an even, positive integer.

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* A function $f(x)$ belongs to A (α, a), where $0 < \alpha \leqslant \pi$, $a < \frac{1}{2}$ if (i) it is an analytic function of $x = re^{i\theta}$ regular in the angle defined by $r > 0$, $|\theta| < \alpha$ and (ii) it is $\mathrm{O}(|x|^{-a-\epsilon})$ for small x and $\mathrm{O}(|x|^{a'-1+\epsilon})$ for large x, for every positive ϵ and uniformly in any angle $|\theta| \leqslant \alpha - \eta < \alpha$.

Also putting, $\omega'(S) = \dfrac{2^{\frac{\mu}{2}+\frac{\theta}{4}-\frac{5}{4}}}{\Gamma\left(\frac{1}{4}+\frac{\nu}{2}+\frac{s}{2}\right)\Gamma\left(\frac{3}{4}+\frac{\nu}{2}-\frac{s}{2}\right)}$

we get the function,[6]

$$f_2(x) = x^{\frac{1}{2}[\mu+\theta-1]}\, e^{-\frac{1}{4}x^2}\, W_{\frac{1}{2}+\frac{\mu}{4}+\frac{\theta}{4}-\frac{\nu}{2};\,\frac{\mu}{4}-\frac{\theta}{4}}\left(\tfrac{1}{2}x^2\right), \tag{1·5}$$

Hence, the resultant kernel is given by,

$$g(x) = \int_0^\infty (xy)^{\lambda+\frac{1}{2}}\, e^{-\frac{1}{2}x^2y^2}\, L_{\frac{\nu}{2}-\frac{\mu}{2}}^{\frac{\mu+\lambda}{2}}\left(\tfrac{1}{2}x^2y^2\right)\cdot$$

$$\times y^{\frac{1}{2}[\mu+\theta-1]}\, e^{-\frac{1}{4}y^2}\, W_{\frac{1}{2}+\frac{\mu}{4}+\frac{\theta}{4}-\frac{\nu}{2},\,\frac{\mu}{4}-\frac{\theta}{4}}\left(\tfrac{1}{2}y^2\right) dy$$

Since, $L_n^{\alpha}(x) = \dfrac{\Gamma(\alpha+1+n)}{n!\,\Gamma(\alpha+1)}\, {}_1F_1(-n;\ \alpha+1;\ x)$,

where n is a positive integer or zero; we have,

$$g(x) = \frac{x^{\lambda+\frac{1}{2}}\,\Gamma\left(\frac{\lambda+\nu}{2}+1\right)}{\Gamma\left(\frac{\nu-\mu}{2}+1\right)\Gamma\left(\frac{\lambda+\mu}{2}+1\right)} \int_0^\infty y^{\frac{\mu+\theta}{2}+\lambda}\, e^{-\frac{1}{2}y^2\left(\frac{1}{2}+x^2\right)}$$

$$\times {}_1F_1\left(\frac{\mu-\nu}{2};\ \frac{\lambda+\mu}{2}+1;\ \tfrac{1}{2}x^2y^2\right) W_{\frac{1}{2}+\frac{\mu}{4}+\frac{\theta}{4}-\frac{\nu}{2},\,\frac{\mu}{4}-\frac{\theta}{4}}\left(\tfrac{1}{4}y^2\right) dy$$

$$= \frac{x^{\lambda+\frac{1}{2}}\,\Gamma\left(\frac{\lambda+\nu}{2}+1\right)}{\Gamma\left(\frac{\nu-\mu}{2}+1\right)\Gamma\left(\frac{\mu-\nu}{2}+1\right)} \sum_{n=0}^{\infty} \frac{\Gamma\left(\frac{\mu-\nu}{2}+n\right)\left(\frac{x^2}{2}\right)^n}{\Gamma\left(\frac{\lambda+\mu}{2}+1+n\right)n!}$$

$$\times \int_0^\infty y^{\frac{\mu+\theta}{2}+\lambda+2n}\, e^{-\frac{1}{2}y^2\left(\frac{1}{2}+x^2\right)} W_{\frac{1}{2}+\frac{\mu}{4}+\frac{\theta}{4}-\frac{\nu}{2},\,\frac{\mu}{4}-\frac{\theta}{4}}\left(\tfrac{1}{2}y^2\right) dy$$

a process easily justifiable due to the uniform convergence of the ${}_1F_1$ function.

Putting $\frac{1}{2}y^2 = z$ and using Goldstein's Integral,

$$\int_0^\infty x^{l-1}\, e^{-x\left(a^2+\frac{1}{2}\right)}\, W_{k,m}(x)\, dx = \frac{\Gamma\left(l-m+\frac{1}{2}\right)\Gamma\left(l+m+\frac{1}{2}\right)}{\Gamma(l-k+1)}$$

$$\times {}_2F_1\left(\begin{matrix} l+m+\frac{1}{2},\ l-m+\frac{1}{2} \\ l-k+1 \end{matrix};\ -a^2\right) \left[R\!\left(a^2+1\right) > 0,\ R\!\left(l \pm m+\tfrac{1}{2}\right) > 0\right]$$

we get

$$g(x)=\frac{x^{\lambda+\frac{1}{2}}\,\Gamma\left(\frac{\lambda+\nu}{2}+1\right)2^{\frac{\mu+\theta}{4}+\frac{\lambda}{2}-\frac{1}{2}}}{\Gamma\left(\frac{\nu-\mu}{2}+1\right)\Gamma\left(\frac{\mu-\nu}{2}+1\right)}\times$$

$$\times\sum_{n=0}^{\infty}\frac{\Gamma\left(\frac{\mu-\nu}{2}+n\right)\Gamma\left(\frac{\lambda+\mu+3}{2}+n\right)\Gamma\left(\frac{\lambda+\theta+1}{2}+n\right)x^{2n}}{n!\,\Gamma\left(\frac{\lambda+\mu+2}{2}+n\right)\Gamma\left(\frac{\lambda+\nu+2}{2}+n\right)}\times$$

$$\times\,{}_2F_1\left(\begin{matrix}\frac{\mu+\lambda+3}{2}+n,\ \frac{\lambda+\theta+1}{2}+n;\\ \frac{\lambda+\nu+2}{2}+n;\end{matrix}-x^2\right)$$

for $\quad R\ell\,(\mu+\lambda+3)>0$ and $R\ell\,(\lambda+\theta+1)>0$.

Our theorem at once gives that $g(x)$ is a kernel transforming R_λ into R_θ.

§ 2. *Corollary.*—If we put $\mu=\nu$ in (1.1) and (1.2) we get as a particular case an interesting result due to B. Mohan, that

"The resultant of two R_ν functions is a kernel of the above type."

Example 1.—Consider the functions,[3]

$$f_1(x)=x^{\mu+\frac{1}{2}}\,e^{-\frac{1}{2}x^2}(\mu>-1),\ \text{a } R_\mu \text{ function},$$

$$f_2(x)=x^{\nu+\frac{1}{2}}\,e^{\frac{1}{4}x^2}\,D_{-2\nu}(x)\,(\nu>-1),\ \text{a } R_\nu \text{ function}.$$

The resultant kernel is given by,

$$g(x)=\int_0^\infty y^{\nu+\frac{1}{2}}\,e^{\frac{1}{4}x^2}\,D_{-2\nu}(y)\,.\,(xy)^{\mu+\frac{1}{2}}\,e^{-\frac{1}{2}x^2y^2}\,dy$$

Using the formula

$$D_n(z)\,e^{\frac{1}{4}z^2}=\frac{\Gamma(\frac{1}{2})\,2^{\frac{n}{2}}}{\Gamma\left(\frac{1}{2}-\frac{n}{2}\right)}\,{}_1F_1\left(-\frac{n}{2};\,-\tfrac{1}{2};\,\tfrac{1}{2}z^2\right)+\frac{\Gamma(-\frac{1}{2})\,2^{\frac{n}{2}-\frac{1}{2}}}{\Gamma\left(-\frac{n}{2}\right)}\times$$

$$\times\,{}_1F_1\left(\frac{1}{2}-\frac{n}{2};\,\frac{3}{2};\,\frac{1}{2}z^2\right)$$

and integrating term by term, we get that

$$g(x)=2^{\frac{\mu-\nu}{2}}\,x^{-\nu-\frac{3}{2}}\left[\frac{\Gamma(\frac{1}{2})}{\Gamma(\nu+\frac{1}{2})}\,{}_2F_1\left(\begin{matrix}\nu,\ \frac{\mu+\nu}{2}+1;\\ -\frac{1}{2};\end{matrix}\ x^{-2}\right)+\right.$$

$$\left.+\frac{\Gamma(-\frac{1}{2})}{x\,\Gamma(\nu)}\,{}_2F_1\left(\begin{matrix}\nu+\frac{1}{2},\ \frac{\mu+\nu+3}{2};\\ \frac{3}{2};\end{matrix}\ x^{-2}\right)\right]$$

for $Rl\,(\mu+\nu+2)>0$, is a kernel transforming R_μ into R_ν.

Example 2.—Next, consider the functions,[3]

$$f_1(x)=x^{\frac{1}{2}}\,J_{\frac{\mu}{2}}(\tfrac{1}{2}x^2)\qquad(\mu>-1),\ \text{a } R_\mu \text{ function.}$$

$$f_2(x)=x^{\nu+\frac{1}{2}}\,e^{-\frac{1}{2}x^2}\qquad(\nu>-1),\ \text{a } R_\nu \text{ function.}$$

The resultant kernel is given by,

$$g(x)=\int_0^\infty y^{\nu+\frac{1}{2}}\,e^{-\frac{1}{2}y^2}\,(xy)^{\frac{1}{2}}\,J_{\frac{\mu}{2}}(\tfrac{1}{2}x^2y^2)\,dy$$

$$=2^{\frac{\nu}{2}}\,x^{\frac{1}{2}}\int_0^\infty y^{\frac{\nu}{2}}\,e^{-y}\,J_{\frac{\mu}{2}}(x^2y)\,dy$$

$$=2^{\frac{\nu}{2}}\,x^{\mu+\frac{1}{2}}\,{}_2F_1\left(\begin{matrix}\frac{\mu+\nu}{4}+\frac{1}{2},\ \frac{\mu+\nu}{4}+1;\\ \frac{\mu}{2}+1;\end{matrix}\ -x^4\right)$$

for $Rl\,(\mu+\nu+2)>0$ and $|x|<1$, is a kernel transforming R_μ into R_ν.

I am deeply indebted to Dr. R. S. Varma for his kind guidance in the preparation of this paper.

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CHEMICAL EXAMINATION OF THE SEEDS OF *CARTHAMUS OXYCANTHA*

Part I. The Component Fatty Acids and the Glyceride Structure of the Seed Oil

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The following investigation describes the component fatty acids and the glyceride structure of the seed oil of *Carthamus oxycantha*. The plant belongs to the natural order compositeæ.

It is distributed throughout the Punjab, Baluchistan, Afghanistan and westwards towards Caucasus. It is called Wild Safflower in English and Poli or Polian in Hindustani. The seeds contain a large quantity (27%) of oil which is used as a medicine in the Punjab (Stewart),[1] and is the chief ingredient of Afridi Wax Works.

Crossley and Le Sueur[2] also have done some work and they report that the oil from seeds is used for edible purposes but add a note of caution: "It is reported that the plant is a troublesome weed in India and this should be carefully considered before introducing it in other countries."

The plant seems to have been discovered very recently as it has not been mentioned in any of the old works and as a result of this very little work has been done on it, though many other plants of this family have been worked out thoroughly, one of them, *Helianthus annus*,[3] having been investigated by us very recently.

Under these circumstances the work was undertaken and the seed oil of this plant thoroughly examined. The oil contains five acids, Myristic, Palmitic, Stearic, Oleic and Linoleic and seven glycerides, though the maximum number of glycerides obtainable from five acids and the glyceryl radical, CH_2–CH–CH_2, is seventy-five.

Vidyarthi[4] has examined the seed oil of *Carthamus tinctorius* and his results are given here for the comparative study of these two plants. He obtained Myristic acid (with Lauric and lower acids) 1·5%, Palmitic 3%, Stearic 1%, Arachidic (with a trace of Lignoceric) 0·5%, Oleic 33% and

Linoleic (with a trace of Linolenic) 61·0%; and the glycerides were Myristo-oleo-linolein 2%, Myristo-di-lionolein 1%, Palmito-oleo-linolein 7%, Palmito-di-linolein 4%, Stearo-oleo-linolein 2%, Stearo-di-linolein 1%, Di-oleo-linolein 15%, Oleo-di-linolein 63% and Tri-linolein 3%. The glycerides of higher acids than Stearic have been considered with Stearic acid and those lower than Myristic with Myristic acid.

On comparing these two oils we see that both of them contain the same component acids as well as the same solid acid contents, but while in *Carthamus oxycantha* the major acid amongst the liquid acids is Oleic acid, in *Carthamus tiontorius* it is Linoleic.

Experimental

A preliminary examination of the seeds of *Carthamus oxycantha* collected near Lyallpur through the kindness of Dr. J. C. Luthra, Principal, Agricultural College, Lyallpur, gave the following results:

Yield of the oil on extraction	27·0%
Average weight of a seed	0·0095 gm.
Ash content of the seeds	2·88%
Water-soluble in the ash	32·32%
Water-insoluble in the ash	67·68%

The qualitative analysis of the ash gave:

Water-soluble: Sodium, Potassium, Carbonate and Chloride.
Water-insoluble: Aluminium, Calcium, Carbonate and Sulphate.

Fifty gms. of the seeds were subjected to extraction by different solvents in succession in a Soxhlet apparatus and the result was as follows:

1.	Petroleum-ether (40°–60°) ..	A light yellow oil. Yield	27.0%
2.	Absolute ether ..	,, ,,	1·7%
3.	Chloroform	Light yellow semi-solid. Yield	0·7%
4.	Ethyl acetate ..	,, ,,	1·7%
5.	Absolute alcohol ..	Pale yellow semi-solid. Did not give any reaction for sugar or glucoside. Yield	0·9%
6.	70% Alcohol ..	,, ,,	0·7%

The expressed and the extracted oils were purified by animal charcoal and Fuller's earth and the physical and chemical constants determined. These results together with those of Crossley and Le Sueur[2] and Barnes and Single[5] are given in Table I below:

TABLE I

Constants	Extracted oil	Expressed oil	Crossley and Le Sueur	Barnes and Single
Saponification Value ..	193·6	195·0	189·4	174·2
Iodine Value (Hanus) ..	112·0	112·8	135·5	167·4
Thiocyanogen Value (24 hrs.) ..	79·6	80·2	..	..
Acid Value ..	1·2	1·34	..	..
Acetyl Value ..	2·75	2·98	..	..
Hehner Value ..	94·8%	93·8%	..	..
Unsaponifiable matter ..	..	0·42%	..	..
Specific Gravity (35° C.) ..	0·9131	0·9175	..	0·9270 (15·5° C.)
Refractive Index (30° C.) ..	1·4697	1·4715	..	1·4755 (20° C.)
R. M. Value ..	0·69	0·84	..	0·60

One kg. of the expressed oil was saponified with alcoholic caustic soda, unsaponifiable matter removed with ether and the mixed fatty acids liberated. The chemical constants of the mixed acids were determined which are given in Table II below:

TABLE II

Constants	Result
Saponification Value ..	200·6
Iodine Value (Hanus) ..	118·7
Thiocyanogen Value (24 hrs.) ..	84·5

The mixed fatty acids were separated into their solid and liquid constituents by Hilditch's modification of Twitchell's lead-salt-alcohol process[6]. The various chemical constants of solid and liquid fractions were found out and are given in Table III below:

TABLE III

Constants	Solid acids	Liquid acids
Percentage ..	8·05	91·95
Saponification Value ..	208·4	199·8
Iodine value (Hanus) ..	4·8	126·0
Thiocyanogen Value (24 hrs.) ..	2·9	90·1

EXAMINATION OF LIQUID ACIDS

2·59 gm. of liquid acids were brominated[7, 8] at −10° C. in dry ether and kept for two hours. No solid separated showing the absence of linolenic

acid. The excess of bromine was destroyed with a solution of sodium thiosulphate, washed with distilled water, dried and the solvent distilled off. Dissolved the residue in 500 c.c. of light petroleum and kept in refrigerator overnight. Some solid separated which was filtered on a weighed Gooch. The filtrate was concentrated to half its bulk and kept again in refrigerator. Some more solid separated which also was transferred to the same Gooch. Solvent from the filtrate was distilled off and the bromine content of the residue determined. The results of the experiment and the calculations are given below in Table IV.

TABLE IV

Weight of acid brominated	..	2·59 gm.
Weight of linoleic acid tetrabromide	..	1·992 ,,
M. P. of tetrabromide	..	113·0° C.
Weight of residue (di- and tetrabromide)	..	2·659 ,,
Bromine content of the residue	..	37·55%
Weight of linoleic acid tetrabromide in residue	..	0·207 ,,
Total weight of tetrabromide	..	2·199 ,,
Weight of oleic acid dibromide in residue	..	2·452 ,,
Weight of linoleic acid	..	1·026 ,,
Weight of oleic acid	..	1·564 ,,
Percentage of linoleic acid in liquid acids	..	39·6
Percentage of oleic acid in liquid acids	..	60·4

Here below in Table V we have given the amounts of saturated, oleic and linoleic acids as obtained from different experiments. We find that they are in close agreement with each other, thus proving the accuracy of our experiments:

TABLE V

Method		Solid acids	Oleic acid	Linoleic acid
		%	%	%
1 I. V. and SCN. V. of mixed acids	..	6·31	55·94	37·75
2 I. V. and SCN. V. of solid and liquid acids	..	7·89	55·51	36·60
3 Bromination of liquid acids	..	..	56·11	36·67
4 Bertram's method	..	7·98	..	..
5 Average of 1 to 4	..	7·39	55·80	36·81
6 From glyceride structure	..	7·26	54·98	37·76

EXAMINATION OF SOLID ACIDS

The solid acids were converted into methyl esters and 70·88 gm. of the esters were fractionally distilled at reduced pressure. The results of the experiment and the calculations[9] are given in Tables VI and VII below.

TABLE VI

Fraction No.	Pressure	Temperature range	Weight in grams
S_1 ..	10 m.m.	170°–175°	4·91
S_2 ..	do	175°–180°	4·95
S_3 ..	do	180°–185°	4·51
S_4 ..	do	185°–190°	7·34
S_5 ..	do	190°–195°	6·80
S_6 ..	do	195°–200°	5·17
S_7 ..	do	200°–205°	7·79
S_8 ..	do	205°–210°	3·75
S_9 ..	do	210°–215°	5·51
S_{10} (residue) ..	do	..	17·15
Loss ..			0·56
Total ..			70·88

Fractions S_1 & S_2—On repeated crystallisation of the liberated acids from dilute acetone, Myristic acid, m.p. 56° C. and Palmitic acid, m.p. 61°–62° C. were obtained. The melting points were not depressed by adding authentic sample of the respective acids.

Fractions S_3, S_4 & S_5—On a similar treatment gave Palmitic acid, m.p , 61°-62° C., which was not depressed by its authentic sample.

Fractions S_6, S_7 & S_8—On a similar treatment gave Palmitic acid, m.p., 61°-62° C. and Stearic acid, m.p., 68°-69° C., which were not depressed by the authentic samples of the respective acids.

Fractions S_9 & S_{10}—Similarly gave Stearic acid, m.p., 68°-69° C., which was not depressed by an authentic sample of the acid.

TABLE VII

Fraction	I.V.	S.E.	Myristic acid		Palmitic acid		Stearic acid		Liquid acids	
			%	gm.	%	gm.	%	gm.	%	gm.
S_1 ..	1·03	253·6	56·67	2·753	37·58	1·846	..	..	0·82	0·040
S_2 ..	1·08	257·5	43·03	2·130	50·65	2·507	..	..	0·86	0·043
S_3 ..	1·27	267·5	8·99	0·405	84·76	3·823	..	..	1·00	0·044
S_4 ..	1·28	268·9	4·66	0·342	89·08	6·539	..	..	1·0	0·073
S_5 ..	1·15	270·1	..	..	93·83	6·533	..	..	0·91	0·062
S_6 ..	1·21	283·7	..	..	48·26	2·496	45·82	2·369	0·96	0·050
S_7 ..	1·61	289·2	..	..	29·06	2·263	64·82	5·049	1·28	0·100
S_8 ..	1·64	289·8	..	..	27·71	1·040	66·15	2·480	1·30	0·049
S_9 ..	2·49	298·2	..	..	..	..	93·33	5·142	1·98	0·109
S_{10} ..	2·24	298·9	..	..	..	..	93·53	16·440	1·78	0·313
				5·630		27·047		31·480		0·883

	On solid acids	On total acids
	%	%
Myristic acid ..	9·02	0·66
Palmitic acid ..	41·93	3·11
Stearic acid ..	49·05	3·62

Five grams of the mixed acids were oxidised by Bertram's method[10] and 7·98% of the solid acids were obtained.

Thus the seed oil of *Carthamus oxycantha* contains Myristic, Palmitic, Stearic, Oleic and Linoleic acids. The percentages are given in Table VIII:

TABLE VIII

		%
Myristic acid	..	0·66
Palmitic acid	..	3·11
Stearic acid	..	3·62
Oleic acid	..	55·80
Linoleic acid	..	36·81

EXAMINATION OF UNSAPONIFIABLE MATTER

The unsaponifiable matter after recrystallisation from absolute alcohol was found to melt at 135°. The acetyl derivative melted at 121°–122°. The substance gave all the colour reactions of Phytosterol, hence it is Sitosterol present in almost all the vegetable oils.

GLYCERIDE STRUCTURE

The purified oil was neutralised with sodium carbonate, dried over fused calcium chloride and filtered.

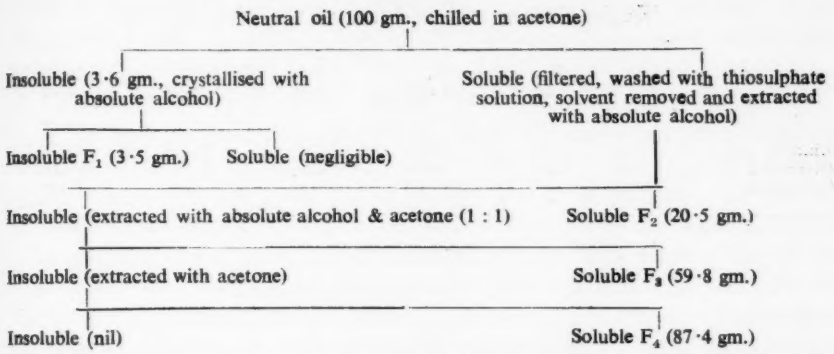
Fifty gm. of the above oil was chilled[11, 12] in the refrigerator for a week with six times its weight of pure and dry acetone. No solid separated showing the absence of tri-saturated and di-saturated-mono-unsaturated glycerides.

The absence of tri-saturated glycerides was further confirmed by oxidising the oil, dissolved in ten times its weight of pure and dry acetone, with four times its weight of powdered potassium permanganate.[13]

One hundred gm. of the oil was brominated[14, 15] in ten times its weight of dry light petroleum at $-5°$ till the brown colour persisted. 3·6 gm. of the solid (F_1) separated which on recrystallisation from absolute alcohol gave a solid, m.p. 78°–79°, and bromine content 51·62%. Therefore F_1 is an impure sample of tetra-bromo linoleic glyceride.

The filtrate after filtering F_1 was treated with sodium thiosulphate solution to destroy excess of bromine, washed and the solvent distilled off. The viscous mass was extracted with absolute alcohol, alcohol and acetone (1 : 1) and acetone in succession. The scheme of separation is given below;

SCHEME


Neutral oil (100 gm., chilled in acetone)
Insoluble (3·6 gm., crystallised with absolute alcohol)
Soluble (filtered, washed with thiosulphate solution, solvent removed and extracted with absolute alcohol)
Insoluble F_1 (3·5 gm.)
Soluble (negligible)
Insoluble (extracted with absolute alcohol & acetone (1 : 1)
Soluble F_2 (20·5 gm.)
Insoluble (extracted with acetone)
Soluble F_3 (59·8 gm.)
Insoluble (nil)
Soluble F_4 (87·4 gm.)

The fractions F_2, F_3 and F_4 were debrominated by taking them in dry methyl alcohol, adding an equivalent amount of zinc dust, saturating with dry hydrochloric acid gas and refluxing for about eight hours. The debrominated fractions were saponified, the unsaponifiable matter removed and the fatty acids liberated. The Iodine, Thiocyanogen and Saponification values of the mixed acids determined. The mixed acids were oxidised with alkaline potassium permanganate[16] and the saponification value of the solid acids thus obtained was also determined. The glyceride structure was calculated with these data as given in Tables IX–XIII below.

TABLE IX

	F_1	F_2	F_3	F_4
Wt. of brominated product in gm. ..	3·5	20·5	59·8	87·4
Wt. of debrominated product in gm. ..	1·69	11·55	3 ·29	51·57
Wt. of unsaponifiable matter in gm. ..	..	0·05	0·13	0·15
Wt. of debrominated product free from unsaponifiable matter	1·69	11·50	35·16	51·42
Mol. % of mixed acids ..	1·69	11·53	35·24	51·54
I. V. of mixed acids ..	..	128·6	115·2	115·21
SCNV. of mixed acids ..	..	80·4	86·2	82·4
S. E. do ..	..	279·6	280·1	282·0
S. E. of solid acids ..	..	260·1	270·5	275·3

TABLE X

Weight per cent. of acids in each fraction

	F_1	F_2	F_3	F_4
Linoleic acid ..	100·0	53·20	32·01	36·21
Oleic acid ..	..	35·81	63·60	55·16
Solid acids ..	..	10·99	4·39	8·63

TABLE XI

Weight per cent. of acids on total acids

	F_1 1·69	F_2 11·53	F_3 35·24	F_4 51·54	Mean 100·0
Linoleic acid ..	1·69	6·13	11·28	18·66	37·76
Oleic acid ..	..	4·13	22·42	28·43	54·98
Solid acids ..	..	1·27	1·54	4·45	7·26

TABLE XII

Mol. per cent. of acids on total acids

	F_1 1·69	F_2 11·53	F_3 35·24	F_4 51·54	Mean 100·0
Linoleic acid ..	1·69	6·09	11·32	18·70	36·80
Oleic acid ..	..	4·08	22·32	28·30	55·70
Solid acids ..	..	1·36	1·60	4·54	7·50

TABLE XIII

Probable glyceride structure

	F_1 1·69	F_2 11·53	F_3 35·24	F_4 51·54	Mean 100·0
1 Tri-saturated glycerides ..	nil	nil	nil	nil	
2 Di-saturated mono-unsaturated glyceride ..	nil	nil	nil	nil	
3 Mono-saturated di-unsaturated					
(*a*) Do oleo-linolein ..	nil	4·08	4·80	13·62	22·50
4 Tri-unsaturated glyceride					
(*a*) Oleo-di-linolein ..	nil*	6·74	nil	4·56	11·30
(*b*) Di-oleo-linolein ..	nil	0·71	29·16	33·36	63·23
(*c*) Tri-olein ..	nil	nil	1·28	nil	1·28
(*d*) Tri-linolein ..	1·69	nil	nil	nil	1·69

N.B.—1. By oxidation of the neutral oil with potassium permanganate in acetone.

2. By chilling the neutral oil in acetone at 0° C.

3 & 4. By calculating from the component fatty acids of the brominated glycerides in the oil.

All the saturated acids have been considered as one acid in the above calculations. According to the law of even distribution we are justified in assuming that the greater portion of the glycerides exist as mono-saturated oleo-linolein rather than mono-saturated di-olein and mono-saturated di-linolein. Further it has been assumed that the solid acids are proportionately distributed in different glycerides. From the above considerations

the probable glyceride structure may be given as Myristo-oleo-linolein 2·05%, Palmito-oleo-linolein 9·82%, Stearo-oleo-linolein 10·33%, Oleo-di-linolein 11·3%, Di-oleo-linolein 62·23%, Tri-olein 1·28% and Tri-linolein 1·69%.

Summary

The fatty oil from the seeds of *Carthamus oxycantha* has been examined and found to have Sp. Gr. (35° C.) 0·9175, Refractive index (30° C.) 1·4715, Acid value 1·34, Sap. val. 195·0, Acetyl val. 2·98, Hehner val. 93·8%, unsaponifiable matter 0·42%, R.M. val. 0·84, Iodine val. (Hanus) 112·8, Thiocyanogen val. (24 hrs.) 80·2. The component fatty acids of the oil are Myristic 0·66%, Palmitic 3·11%, Stearic 3·62%, Oleic 55·80% and Linoleic 36·81%. The unsaponifiable matter is Sitosterol, m.p. 135° C. The component glycerides of the oil are Myristo-oleo-linolein 2·05%, Palmito-oleo-linolein 9·82%, Stearo-oleo-linolein 10·33%, Oleo-dilinolein 11·30%, Di-oleo-linolein 62·23%, Tri-olein 1·28% and Tri-linolein 1·69%.

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CHEMICAL EXAMINATION OF SEEDS OF *RAPHANUS SATIVUS* LINN.

Part I. The Component Fatty Acids and the Probable Glyceride Structure of the Oil

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Raphanus sativus Linn., called Radish in English and Muli in Hindi, belongs to the natural order Cruciferæ. It is an annual plant growing up to an altitude of 16,000 ft. throughout India and in temperate and warm countries. It is one of the most widely known plants and has been used for different diseases and ailments in Ayurvedic and Unani systems of medicine.[1, 2, 3]

Though the plant is so widely known, no work of appreciable importance has been done on it, although exhaustive work on Rape oil, which belongs to the same family, has been done. On comparison of these two oils, we find that in both Erucic acid is a major component of the unsaturated acids. But there is a marked contrast in the amounts. The Erucic and Oleic acid contents of Radish oil are 20% and 60% respectively, while reverse is the case with Rape oil. Further, whereas Behenic acid is the largest constituent of solid acids of Radish oil, Lignoceric acid being absent; in Rape oil, Behenic acid is in minute quantities, the main constituent being Lignoceric acid.

An analysis of the oil has been reported by Grimme.[4] He has worked out some four different samples, the results of which are given in Table I. De Negri and Fabis as well as Crossley and Le Sueur also analysed some samples with the same results.

The present work was undertaken for the study of the glyceride structure of the oil and some sixteen glycerides were found, though the total number of glycerides which may be obtained by any combination of eight different acids and the glyceryl radical, CH_2CH-CH_2, is 288.

EXPERIMENTAL

The material employed for this investigation consisted of authentic seeds of *Raphanus sativus* and the preliminary examination was conducted with the following results:

Average weight of a seed	0·0022 gm.
Ash content of the seeds	3·4%
Water-insoluble in the ash	56·2%
Water-soluble in the ash	43·8%

The qualitative constitution of the ash is:

Water-insoluble: Calcium, Aluminium, Carbonate, Phosphate.

Water-soluble: Potassium, Carbonate.

Fifty gms. of the crushed seeds were extracted in succession with various solvents in a Sohxlet apparatus with the following result:

1. Petroleum ether (40°–60°) .. A yellow oil. Yield 46·5%
2. Absolute ether .. .. „ 2·2%
3. Chloroform .. .. .. A yellow sticky solid. Yield 0·9%
4. Ethyl acetate .. .. „ 0·8%
5. Absolute alcohol .. .. A sticky solid which gave test for glucoside. Yield 1·2%
6. 70% Alcohol .. .. A sticky solid which gave test for glucoside. Yield 1·0%

Examination of the Fatty Oil

2 Kg. of the crushed seeds were extracted with light petroleum and the oil thus obtained was purified with animal charcoal and Fuller's Earth. The physical and chemical constants of the oil were determined as given in Table I below.

Table I

	Constants	Our results	Grimme's results
1	Sp. Gr. (30° C.) ..	·9173	·9165– ·9178 (15·5° C.)
2	Refractive index (30° C.) ..	1·4704	1·4750–1·4722 (20° C.)
3	Acid value ..	·94	..
4	Acetyl value ..	2·83	..
5	R. M. value ..	0·61	..
6	Unsaponifiable matter ..	0·24%	..
7	Hehner value ..	94·2%	94·9%
8	Saponification value ..	178·9	179·6–181·6
9	Iodine value (Hanus) ..	103·1	90·8–103·5
10	Thiocyanogen value (24 hrs.) ..	79·02	..

500 Gm. of the oil was saponified with alcoholic caustic soda, the unsaponifiable matter removed with ether and the mixed fatty acids liberated. The constants of the mixed acids are given in Table II.

TABLE II

Constant	Results
Saponification value ..	183·4
Iodine value (Hanus) ..	104·4
Thiocyanogen value (24 hrs.) ..	86·44

The mixed fatty acids were separated into saturated, erucic and liquid acids by lead-salt alcohol process.[5, 6] On cooling the hot mixtures of mixed acids solution and lead acetate solution, lead salts of solid and erucic acids got deposited, erucic acid salt being pasty. After filtering off the lead salts of liquid acids, the residue was boiled with 95% alcohol for recrystallisation, when the solid acid salts formed the upper layer of a fluffy precipitate, erucic acid salt remaining stuck to the bottom of the vessel. The lead salts of solid acids were transferred to a filter paper and washed with alcohol, the erucic acid salt remained stuck in the vessel. The different fractions thus separated were converted into respective acids and their constants determined which are given below in Table III.

TABLE III

Constants	Solid acids	Erucic acid	Liquid acids
Percentage ..	8·98	22·04	68·98
Saponification value ..	179·9	167·3	197·6
Saponification equivalent ..	311·8	335·4	283·9
Iodine value (Hanus) ..	3·6	78·12	105·8
Thiocyanogen value (24 hrs.) ..	2·7	75·23	94·86

EXAMINATION OF LIQUID ACIDS

2·676 gm. of liquid acids were brominated[7, 8] at −10° C. in dry ether and kept for two hours. Some Linolenic acid hexabromide separated which was filtered on a weighed Gooch. Excess of bromine in the filtrate was destroyed with sodium thiosulphate solution, washed, dried and the solvent distilled off. The residue was dissolved in 500 c.c. of light petroleum and kept in refrigerator overnight. No solid separated and the solvent was distilled off. The bromine content of the residue was determined and the results of bromination and the calculation are given in Table IV.

The percentages of different acids as calculated by different methods are given in Table V. The percentage weights agree fairly closely though the solid acids as obtained by glyceride structure method are somewhat higher than those obtained by determination of component acids.

TABLE IV

Weight of acid brominated	..	2·676 gm.
Weight of linolenic acid hexabromide	..	·5618 gm.
M. P. of hexa-bromide	..	175–176° C.
Weight of residue (di- and tetrabromide)	..	3·4578 gm.
Bromine content of the residue	..	38·06%
Weight of linoleic acid tetrabromide in residue	..	·3728 gm.
Weight of oleic acic dibromide in residue	..	3·0850 gm.
Weight of linolenic acid	..	·1338 gm.
Weight of linoleic acid	..	·1740 gm.
Weight of oleic acid	..	1·9682 gm.
Linolenic acid in liquid acids	..	5·0%
Linoleic acid in liquid acids	..	6·5%
Oleic acid in liquid acids	..	88·5%

TABLE V

Method		Linolenic acid %	Linoleic acid %	Oleic acid %	Erucic acid %	Solid acid %
1 Twitchell's method	..	←	68·98	→	22·04	8·98
2 I.V. and SCN. V. of liquid acids	..	3·84	4·53	60·61	..	..
3 By bromination of liquid acids	..	3·45	4·48	61·05	..	..
4 Average of 1 to 3	..	3·65	4·50	60·83	22·04	8·98
5 By glyceride structure	..	2·07	5·22	57·97	21·59	13·15

EXAMINATION OF SOLID ACIDS

The solid acids were converted into their methyl esters and 21·24 gm. of these esters were fractionally distilled at reduced pressure. The results of distillation and calculation[9] are given in Tables VI and VII.

TABLE VI

Fraction No.		Pressure	Temperature Range	Weight in grams
S_1	..	10 m.m.	185°–190° C.	2·54
S_2	..	,,	190°–195° C.	3·60
S_3	..	,,	195°–200° C.	7·08
S_4	..	,,	200°–205° C.	2·53
S_5 (residue)	..	..	..	5·15
Loss	..			·34
Total	..			21·24

Fractions S_1 & S_2—By repeated crystallisation from dilute acetone of the liberated acids gave Palmitic acid m.p. 61°–62° C., and Stearic acid, m.p. 68°–69° C., which were not depressed by adding authentic samples of the respective acids.

Fractions S_3 & S_4—Similarly gave Arachidic acid, m.p. 76°–77° C., and Behenic acid, m.p. 81°–82° C.

Fraction S_5—Similarly gave Behenic acid, m.p. 81°–82° C. Arachidic and Behenic acids needed confirmation for want of authentic samples of these acids, but on mixing the same acid from different fraction, the m.p. was not lowered. The saponification equivalents of these acids were 315·0 and 335·0 respectively.

TABLE VII

Fraction	I.V.	S.E.	Palmitic acid		Stearic acid		Arachidic acid		Behenic acid		Liquid acids	
			%	gm.	%	gm.	%	gm.	%	gm.	%	gm.
S_1 ..	1·09	278·4	67·57	1·716	27·27	·692	..	..	..	..	·95	·024
S_2 ..	·96	289·2	29·50	1·062	64·73	2·330	..	..	..	..	·91	·033
S_3 ..	·99	330·7	..	..	..	..	76·79	5·439	18·01	1·270	·94	·067
S_4 ..	1·25	340·6	..	..	..	..	42·21	1·068	52·50	1·328	1·18	·030
S_5 ..	1·49	353·5	..	..	..	..	..	..	94·62	4·873	1·41	·072
				2·778		3·022		6·507		7·471		·226

	On solid acids %	On mixed acids %
Palmitic acid ..	14·04	1·26
Stearic acid ..	15·32	1·38
Arachidic acid ..	32·88	2·96
Behenic acid ..	37·76	3·38

The component acids of the oil from the seeds of *Raphanus sativus*, therefore, consist of Palmitic, Stearic, Arachidic, Behenic, Erucic, Oleic, Linoleic and Linolenic acids. The percentage of these acids are given in Table VIII below.

TABLE VIII

	Weight %
Palmitic acid ..	1·26
Stearic acid ..	1·38
Arachidic acid ..	2·96
Behenic acid ..	3·38
Erucic acid ..	22·04
Oleic acid ..	60·83
Linoleic acid	4·50
Linolenic acid ..	3·65

EXAMINATION OF UNSAPONIFIABLE MATTER

The unsaponifiable matter extracted from the soap solution, on recrystallisation from absolute alcohol melted at 134°–135°. The m.p. of acetyl derivative was 121° and it responded to all the colour[10] reactions of phytosterol, hence we conclude it to be Sitosterol.

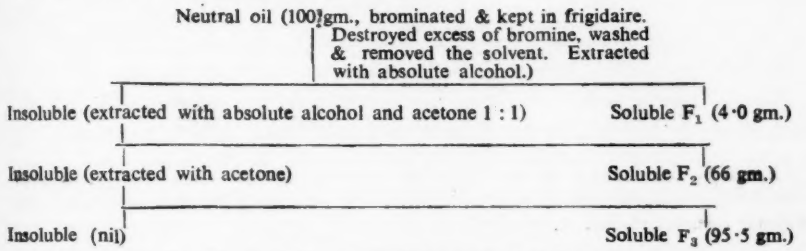
THE COMPONENT GLYCERIDES

The purified oil was dried over fused calcium chloride and neutralised with sodium carbonate and filtered,

Fifty gm. of the oil was chilled[11, 12] in the refrigerator with six times its weight of pure and dry acetone for a week. No solid was deposited showing the absence of tri-saturated and di-saturated mono-unsaturated glycerides.

Fifty gm. of the oil was oxidised with four times its weight of powdered potassium permanganate[13] taken in ten times its weight of dry acetone. On repeating the process nothing remained confirming the absence of tri-saturated glycerides.

One hundred gm. of the oil was brominated[14, 15] in ten times its weight of dry light petroleum and kept in the frigidaire overnight. Excess of bromine was destroyed with sodium thiosulphate solution, washed, dried and the solvent distilled off. The viscous residue was extracted with absolute alcohol, absolute alcohol and acetone (1 : 1) and acetone in succession. The scheme of separation is given below.


Neutral oil (100 gm., brominated & kept in frigidaire. Destroyed excess of bromine, washed & removed the solvent. Extracted with absolute alcohol.)
Insoluble (extracted with absolute alcohol and acetone 1 : 1)
Soluble F_1 (4·0 gm.)
Insoluble (extracted with acetone)
Soluble F_2 (66 gm.)
Insoluble (nil)
Soluble F_3 (95·5 gm.)

Fractions F_1, F_2 and F_3 were debrominated by taking them in dry methyl alcohol, adding equivalent amount of zinc dust, saturating with dry hydrochloric acid gas and refluxing for about eight hours. The debrominated products were extracted with light petroleum, saponified, unsaponifiable matter removed and the mixed fatty acids liberated. Iodine

TABLE IX

	F_1	F_2	F_3
Wt. of brominated glyceride in gm. ..	4·0	66·0	95·5
Wt. of debrominated glyceride in gm. ..	1·55	41·17	57·29
Wt. of unsaponifiable matter in gm. ..	..	·01	·02
Wt of debrominated glyceride free from unsaponifiable matter	1·55	41·16	57·27
Mol. % of mixed acids ..	2·64	40·69	56·67
I. V. of mixed acids ..	270·0	99·45	110·0
SCN. V. of mixed acids ..	175·0	..	..
S.E. of mixed acids ..	..	321·8	321·5
S.E. of solid acids ..	..	322·4	306·8
IVV of liquid acids ..	..	105·6	112·0
SCN.V. of liquid acids ..	..	100·1	102·5

value of all the three fractions, Thiocyanogen value of F_1 and the sap. equivalents of F_2 and F_3 determined. F_2 and F_3 fractions were subjected to lead-salt alcohol process for separating solid, erucic and liquid acids. Sap. equivalents of solid acid and I.V. and SCN.V. of liquid acids were determined. The results of the experiments and calculations are given in Tables IX–XIII.

TABLE X

	F_2	F_3
Solid acids ..	22·57%	6·68%
Erucic acids ..	21·44%	22·31%
Liquid acids ..	55·99%	71·01%

TABLE XI

Percentage of acids in liquid acids

	F_1	F_2	F_3
Linolenic acid ..	100·0	..	..
Linoleic acid ..	..	6·07	10·48
Oleic acid ..	..	93·93	89·52

N.B.—In F_1 the amounts of the other two acids being too small, they have been considered with Linolenic acid while in F_2 and F_3 the amount of linolenic acid being too small it has been included in linoleic acid.

TABLE XII

Weight percentage of acids in mixed acids

	F_1	F_2	F_3
Linolenic acid ..	100·0	..	..
Linoleic acid ..	..	3·40	7·44
Oleic acid ..	..	52·59	63·57
Erucic acid ..	..	21·44	22·31
Solid acids ..	..	22·57	6·68

TABLE XIII

Mol. percentage of acids on total acids

	F_1 2·64	F_2 40·69	F_3 56·67	Mean 100
Linolenic acid ..	2·64	..	..	2·64
Linoleic acid ..	..	1·49	4·46	5·95
Oleic acid ..	..	22·86	37·58	60·44
Erucic acid ..	..	7·81	11·00	18·81
Solid acids ..	..	8·53	3·63	12·16

TABLE XIV

Probable glyceride structure

	F_1 2·64	F_2 40·69	F_3 59·67	Mean 100·00
1 Trisaturated glycerides ..	..	..	..	
2 Disaturated monounsaturated glycerides ..	..	..	..	
3 Monosaturated diunsaturated—				
(*a*) do oleic-linolein ..	nil	2·25	3·63	5·88
(*b*) do erucio-linolein ..	nil	2·25	3·63	5·88
(*c*) do erucio-olein ..	nil	21·09	3·63	24·72
4 Triunsaturated glycerides—				
(*a*) Erucio-linoleo-olein ..	nil	nil	6·12	6·12
(*b*) Erucio-diolein ..	nil	·09	19·62	19·71
(*c*) Triolein ..	nil	15·01	20·04	35·05
(*d*) Trilinolenin ..	2·64	nil	nil	2·64

1. By oxidising the neutral oil dissolved in pure and dry acetone with powdered potassium permanganate.

2. By chilling the neutral oil dissolved in pure and dry acetone at 0° C.

3 & 4. By calculating from the component fatty acids of the brominated glyceride in the oil.

All the saturated acids have been considered as one acid in these calculations. In view of the fact that the acids distribute themselves evenly, we are justified in assuming that the glycerides exist as complex glycerides to the greatest extent possible. The simple glycerides are formed when no other alternative is left. It is further assumed that the solid acids are proportionately divided. Thus from the above considerations the probable glyceride structure of the oil is Palmito-oleo-linolein ·99%, Palmito-erucio-linolein ·99%, Stearo-oleo-linolein ·98%, Stearo-erucio-linolein ·98%, Arachido-oleo-linolein 1·91%, Arachido-erucio-linolein 1·91%, Beheno-oleo-linolein 2·00%, Beheno-erucio-linolein 2·00%, Palmito-erucio-olein 4·18%, Stearo-erucio-olein 4·10%, Arachido-erucio-olein 8·03%, Beheno-erucio-olein 8·41%, Erucio-oleo-linolein 6·12%, Erucio-diolein 19·71%, Triolein 35·05% and trilinolenin 2·64%.

SUMMARY

The fatty oil from the seeds of *Raphanus sativus* Linn. has been examined and found to have Sp. Gr. (30° C.) ·9173, Refractive index (30° C.) 1·4704, Hehner val. 94·2%, R. M. Val. ·61, Acid val. ·94, Acetyl val. 2·83. Unsaponifiable matter ·24%, I.V. (Hanus) 103·1, SCN.V. (24 hrs.) 79·02, Sap. val. 178·5. The component fatty acids of the oil are Palmitic 1·26%, Stearic 1·39%, Arachidic 2·96%, Behenic 3·38%, Linolenic 3·65%, Linoleic 4·50%, Oleic 60·38% and Erucic 22·04%. The component glycerides of

the oil are Palmito-oleo-linolein ·99%, Palmito-erucio-linolein ·99%, Palmito-erucio-olein 4·18%, Stearo-oleo-linolein ·98%, Stearo-erucio-linolein ·98%, Stearo-erucio-olein 4·10%, Arachido-oleo-linolein 1·91%, Arachido-erucio-linolein 1·91%, Arachido-erucio-olein 8·03%, Beheno-oleo-linolein 2·00%, Beheno-erucio-linolein 2·00%, Beheno-eurucio-olein 8·41%, Erucio-oleo-linolein 6·12%, Erucio-diolein 19·71%, Triolein 35·05% and Trilinolenin 2·64%. The unsaponifiable matter is Sitosterol, m.p. 135° C.

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THE DISPERSION FORMULÆ OF THE ALKALI HALIDES

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1. Introduction

The alkali halides are transparent from about 2000 Å in the ultra-violet to the near infra-red region, and some of them like sylvine, rocksalt and potassium bromide possess a far infra-red transparency extending to about 25 μ. The refractive indices of all of them have been determined from their transmission limits in the ultra-violet to their transmission limits in the infra-red or at least to the red end of the visible region. The absorption in the ultra-violet has been found to be due to discrete eigenfrequencies which increase in number with increasing mass of the metal and the halogen. Recent theoretical work in this laboratory has shown that the infra-red absorption of the alkali halides is due to nine fundamental frequencies, their overtones and combinations. No systematic attempt has so far been made to correlate the dispersion and absorption data by means of dispersion formulæ. Such formulæ are important because they provide a clue to the strength of the various absorptions and to the nature of the polarisation field within the substance, and constitute the first step in predicting its thermo-optic behaviour. In the present paper the author has attempted to give dispersion formulæ expressing the refractive indices of the alkali halides in terms of their characteristic absorptions.

2. The Data

Following Ramachandran (1947) the author has summarised in Table I the data on the refractive indices. All the measurements except those of Martens are correct only to one unit in the third place of decimals while Martens has achieved an accuracy of one unit in the fourth, and possibly the fifth place.

The ultra-violet absorption has been studied by Hilsch and Pohl (1930) up to 1600 Å and by Schneider and O'Bryan (1937) up to 1050 Å. In the infra-red, the nine fundamental frequencies cluster together in groups, giving rise to two or three broad peaks of intense absorption. But the infra-red dispersion data are not sufficiently precise to permit the representation of all these nine. It is found that in regions of little or no absorption, the

Compound	Reference	Wave-lengths for which measurements are available (microns)
LiF ..	Gyulai (1927)	0·193 to 0·578
	Hohlo (1937)	0·546 to 55·0
NaF ..	Kublitzky (1934)	0·186 to 0·578
	Hohlo (1937)	0·546 to 55·0
KF ..	Kublitzky (1934)	0·214 to 0·578
KCl ..	Martens (1901)	0·185 to 0·768
	Paschen (1908)	0·589 to 17·168
RbCl ..	Gyulai (1927)	0·193 to 0·577
KBr ..	Gyulai (1927)	0·206 to 0·577
	Gundelach (1939)	0·768 to 28·5
RbBr ..	Kublitzky (1934)	0·219 to 0·578
RbI ..	Kublitzky (1934)	0·253 to 0·578

infra-red contribution to the refractive index is fairly well given by a single term situated in the region of maximum absorption.

3. The Formulæ Proposed

It has been shown by Ramachandran that a dispersion formula of the Drude type best fits the alkali halides. Consequently, the author has adhered (except in the case of potassium fluoride) to the Drude formula

$$n^2 = 1 + \sum_r \frac{b_r \lambda^2}{\lambda^2 - \lambda_r^2},$$

where the λ_r's are the wave-lengths of the characteristic absorptions, with strengths b_r. Putting $\lambda = \infty$, we have

$$n_\infty^2 = 1 + \sum_r b_r$$

that is, the expression on the right-hand side should give the dielectric constant for static fields. The actual formulæ are given below:—

Fluorides

(1) *Lithium Fluoride*

$$n^2 = 1 \cdot 34880 + \frac{0 \cdot 57862\,\lambda^2}{\lambda^2 - 0 \cdot 00814} + \frac{7 \cdot 34258\,\lambda^2}{\lambda^2 - 1106 \cdot 6}$$

Dielectric constant (experimental) = 9·27

„ (formula) = 9·27

Infra-red absorption (experimental) band from 16 μ to 38 μ with peak at 32·6 μ

„ (formula) = 33·3 μ

Ultra-violet absorption (experimental) : no data, absorption starts from 1100 Å.U.

„ (formula) : 902 Å.U.

Wave-length (microns)	Calculated n	Observed n	Wave-length (microns)	Calculated n	Observed n
0·1935	1·4450	1·4450	3·30	1·3619	1·3619
0·2026	1·4389	1·4390	5·70	1·3056	1·3058
0·2194	1·4300	1·4300	8·88	1·168	1·173
0·3126	1·4069	1·4070	10·82	1·028	1·022
0·4358	1·3968	1·3973	11·62	0·951	0·952
0·5461	1·3931	1·3929	13	0·759	0·75
			13·5	0·682	0·65
0·80	1·3895	1·3880	14·0	0·589	0·50
			14·5	0·453	0·40
			15	0·232	0·20

(2) *Sodium Fluoride*

$$n^2 = 1\cdot42664 + \frac{0\cdot32052\,\lambda^2}{\lambda^2 - 0\cdot013056} + \frac{4\cdot25284\,\lambda^2}{\lambda^2 - 2044\cdot8}$$

Dielectric constant (experimental) = 6·00

„ (formula) = 6·00

Infra-red absorption (experimental) = band from 25 μ to 48 μ with peak at 41 μ

„ (formula) : 45 μ

Ultra-violet absorption (Experimental) : 1170 Å.

„ (formula) : 1140 Å

Wave-length (microns)	n (calculated)	n (experimental)	Wave-length (microns)	n (calculated)	n (experimental)
0·18629	1·3930	1·3930	0·5461	1·3255	1·3260
0·20255	1·3772	1·3772	0·80	1·323	1·323
0·30215	1·3417	1·3417	2·00	1·319	1·318
0·43583	1·3306	1·3300	5·10	1·301	1·301
			10·10	1·235	1·231

(3) *Potassium Fluoride*

$$n^2 = 1\cdot84782 + \frac{0\cdot004350}{\lambda^2 - 0\cdot015876} - 0\cdot3017\,\lambda^2$$

No infra-red data

Ultra-violet absorption (experimental) : 1260 Å

„ (formula) : 1260 Å

Wave-length (microns)	n (calculated)	n (experimental)	Wave-length (microns)	n (calculated)	n (observed)
0·2144	1·4111	1·4111	0·4358	1·3664	1·3661
			0·5461	1·3617	1·3617
0·2749	1·3852	1·3855	0·578	1·361	1·3610

Chlorides

(1) *Rocksalt*

A formula on these lines has already been worked out by G. N. Ramachandran. It is included here for the cake of completeness.

$$n^2 = 1 + \frac{0\cdot 187895\,\lambda^2}{\lambda^2-(0\cdot 0500)^2} + \frac{0\cdot 497649\,\lambda^2}{\lambda^2-(0\cdot 1000)^2} + \frac{0\cdot 384897\,\lambda^2}{\lambda^2-(0\cdot 1280)^2} + \frac{0\cdot 259500\,\lambda^2}{\lambda^2-(0\cdot 1580)^2} + \frac{3\cdot 470\,\lambda^2}{\lambda^2-(61\cdot 1)^2}$$

(2) *Sylvine*

$$n^2 = 1\cdot 243412 + \frac{0\cdot 357362\,\lambda^2}{\lambda^2-0\cdot 01000} + \frac{0\cdot 37616\,\lambda^2}{\lambda^2-0\cdot 017161} + \frac{0\cdot 198086\,\lambda^2}{\lambda^2-0\cdot 2\,\cdot 244} + \frac{2\cdot 514254\,\lambda^2}{\lambda^2-4998\cdot 5}$$

Dielectric constant (experimental) = 4·68
" (formula) = 4·69
Infra-red absorption (experimental) = band with peak at 70·7 μ
(formula) = 70·7 μ
Ultra-violet absorption (experimental) = 1000, 1310, 1620 Å
" (formula) = 1000, 1310, 1620 Å

Wave-length (microns)	n (calculated)	n (observed)	Wave-length (microns)	n (calculated)	n (observed)
0·185409	1·82650	1·82704	1·003	1·4799	1·4799
0·22400	1·63606	1·63606	2·974	1·4739	1·4742
0·30827	1·54130	1·54130	5·137	1·4705	1·4706
0·358702	1·52109	1·52109	7·080	1·4662	1·4660
0·410185	1·50901	1·50901	11·197	1·4527	1·4522
0·508660	1·49612	1·49614	18·10	1·4135	1·4108
0·546073	1·49311	1·49313	22·50	1·373	1·369
0·62784	1·48841	1·48841			
0·76824	1·48366	1·48374			

(3) *Rubidium Chloride*

$$n^2 = 1\cdot 4752 + \frac{55511\,\lambda^2}{\lambda^2-0\cdot 019044} + \frac{0\cdot 15241\,\lambda^2}{\lambda^2-0\cdot 027556} - 0\cdot 0004\,\lambda^2$$

No infra-red data
Ultra-violet absorption (observed) — 1380, 1660 Å
" (formula) — 1380, 1660 Å

Wave-length (microns)	n (calculated)	n (observed)	Wave-length (microns)	n (calculated)	n (observed)
0·1935	1·7839	1·7839	0·4046	1·5122	1·5123
0·2063	1·7065	1·7068	0·4916	1·4999	1·4999
0·2265	1·6 93	1·6387	0·5461	1·4953	1·4951
0·3341	1·5319	1·5322			

Bromides

(1) *Potassium Bromide*

$$n^2 = 1{\cdot}4725 + \frac{0{\cdot}6204\,\lambda^2}{\lambda^2 - 0{\cdot}021318} + \frac{0{\cdot}2674\,\lambda^2}{\lambda^2 - 0{\cdot}033124} + \frac{2{\cdot}2847\,\lambda^2}{\lambda^2 - 7365{\cdot}7}$$

Dielectric constant (experimental) = 4·78
,, (formula) = 4·64
Infra-red absorption (experimental) = band with peak at 88 μ
,, (formula) = 86 μ
Ultra-violet absorption (experimental) = 1460, 1730, 1880 Å
,, (formula) = 1460, 1820 Å

Wave-length (microns)	n (calculated)	n (observed)	Wave-length (microns)	n (calculated)	n (observed)
0·2144	1·8934	1·8934	2·357	1·5359	1·5367
0·2483	1·7317	1·7317	3·536	1·5353	1·5350
0·2536	1·7181	1·7181	4·714	1·5344	1·5339
0·4358	1·5790	1·5802	5·893	1·5332	1·5325
0·5461	1·5625	1·5625	8·250	1·5296	1·5290
0·6152	1·5566	1·5559	10·018	1·5265	1·5255

(2) *Rubidium Bromide*

$$n^2 = 1{\cdot}4500 + \frac{0{\cdot}2000\,\lambda^2}{\lambda^2 - 0{\cdot}015129} + \frac{0{\cdot}3651\,\lambda^2}{\lambda^2 - 0{\cdot}022650} + \frac{0{\cdot}3224\,\lambda^2}{\lambda^2 - 0{\cdot}033124}$$

No infra-red data
Ultra-violet absorption (experimental): 1230, 1460, 1550, 1780, 1910 Å
,, (formula) : 1230, 1505, 1820 Å

Wave-length (microns)	n (calculated)	n (observed)	Wave-length (microns)	n (calculated)	n (observed)
0·2195	1·8610	1·8610	0·4358	1·5725	1·5725
0·2537	1·7143	1·7120	0·5461	1·5550	1·5550
0·3341	1·6118	1·6113			

Iodides

(1) *Potassium Iodide*

A formula has already been worked out by Ramachandran.

(2) *Rubidium Iodide*

$$n^2 = 1{\cdot}6017 + \frac{0{\cdot}01749\,\lambda^2}{\lambda^2 - 0{\cdot}015625} + \frac{0{\cdot}83466\,\lambda^2}{\lambda^2 - 0{\cdot}033948} + \frac{0{\cdot}13917\,\lambda^2}{\lambda^2 - 0{\cdot}049729}$$

No infra-red data
Ultra-violet absorption (experimental) : 1200, 1340, 1560 (weak) 1790, 1870, 2230 Å
,, (formula) : 1250, 1840, 2230 Å

Wave-length (microns)	n (calculated)	n (observed)	Wave-length (microns)	n (calculated)	n (observed)
0·25165	1·9956	1·9956	0·40466	1·6930	1·6960
0·27488	1·8788	1·8806	0·47999	1·6657	1·6687
0·30215	1·8019	1·8019	0·54607	1·6515	1·6515

4. *Conclusion*

In the case of RbCl, the bromides and the iodides, the difference between the observed and the calculated values is more than the limit of experimental error, namely one unit in the third decimal place. This is due to the fact that when the number of characteristic frequencies is large, the near ones have to be lumped together in order to make the calculations possible. Also the peaks are broad and diffuse and when they are close to the region of measurement (as in the case of the 2230 Å band for RbI) any slight error in their representation will give rise to a huge error in the refractive index. It is to be noted that the replacement of the nine infra-red frequencies by a single term fails at wave-lengths close to the infra-red bands. The excess over unity in the first term is due to the activity of frequencies in the remote ultra-violet, which varies so little in the region under consideration that it can be considered constant.

I express my thanks to Sir C. V. Raman for suggesting this investigation and for guidance during its course.

Summary

Dispersion formulæ of the Drude form have been worked out using the latest data on dispersion and absorption for all the alkali halides for which measurements are available and for which no formulæ have been proposed so far.

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DIRECTIONAL VARIATIONS OF PHOTOCONDUCTIVITY IN DIAMOND

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1. Introduction

It is well known that the diamonds which are transparent to ultra-violet radiations in the region λ 2250–3000 are conspicuously photo-conducting. Under the polarisation microscope, they exhibit fairly strong birefringence with sets of parallel streaks running in different directions. G. N. Ramachandran (1946) has shown that these streaks are due to the presence of fine laminations inside the crystal which usually occur either along one, two or three of its octahedral planes or along its dodecahedral planes or along both. These laminæ being regions of discontinuity, it is quite likely that thay may have some influence on the photoconducting behaviour of the specimen. Experiments done with five cleavage plates of diamond, *viz.*, N. C. 60, 124, 174, 178 and 181 in Sir C. V. Raman's collection, have shown that, in each of these cases, the conductivity is maximum along the direction of the laminæ and least in the perpendicular direction. It has further been observed that, though they are uniformly transparent to the ultra-violet, in some cases, the conductivity is different in different regions of theirs, the highly birefringent parts being less conducting than the others. The latter phenomenon, it may be mentioned, has already been observed by the author and reported in an earlier paper (K. Achyuthan, 1946).

2. Experimental Technique

Full experimental details as well as the general precautions adhered to have been dealt with in the earlier paper. In order to know the exact direction along which current is flowing through the diamond, a telemicroscope with a graduated disc attached to its eye-piece is focussed on it. By adjusting its cross-wire to lie successively along the electrodes and along any one of the edges of the diamond and noting the difference in the readings of the graduated disc, the direction along which current flows inside the diamond can be ascertained. By rotating the crystal about a horizontal axis, the current can be made to pass along different directions through it.

All the five diamonds studied are cleavage plates of thickness varying from 0·5 mm. to about 1·7 mm. N. C. 60 is a large plate (10 mm./6 mm.) with its faces cleaved along the (211) plane whereas N. C. 124 is a small bit with the cleavage along the (111) plane. N. C. 174 is a thick diamond about 1·7 mm. thick and approximately is in the form of a semicircle, but N. C. 178 is almost circular and also much thinner, its faces being in the (211) plane. N. C. 181, however, is the largest of the lot, its edges being bevelled and slanting.

3. Birefringence Patterns of the Diamonds

The diamond under study is stuck on a glass plate and placed between two crossed polaroids at such an orientation with respect to their axes that all the birefringence streaks are seen clearly with the same setting. Using green light for illumination, a magnified picture of it is taken on a selochrome plate, using a horizontal camera. The photographs of N. C. 178, 174, 124 and 181, thus taken, are reproduced in the plate attached herewith and numbered respectively from 1 to 4. In the case of N. C. 60, the intensity of birefringence varied so much from region to region that it was not possible to take a picture where all the details came out clearly. At the region where the birefringence is very weak, there is one set of streaks which run inclined at a slight angle to the shorter of the edges; whereas at the other regions, the pattern is patchy.

4. Relation between Birefringence and Photoconductivity

Diamond N. C. 178.—This shows such a large variation of conductivity over its area that four separate areas were chosen in it depending upon the nature of birefringence exhibited there; (I) where the birefringence is weak and only the horizontal streaks are present; (II) where it is more intense and two other sets inclined at 60° to each other are faintly seen; (III) where the 60° streaks are more prominent and (IV) where the horizontal streaks are very faint. The polar diagrams drawn to the same scale, representing the conductivity against the orientation, for the four different regions are shown in Fig. 1, curves I–IV, along with a trace of the diamond at the centre. The conspicuously large conductivity of region I and the directional properties of the diamond as a whole stand out prominent. The gradual suppression of the horizontal maximum, as one views the curves from I to IV is also worthy of note.

N. C. 174.—Here too, four regions are chosen depending on the variations in the nature of birefringence, though the variations in this case are not so prominent as in the other. There are two maxima in each case, as seen from Fig. 2 (curves I–IV) one maximum predominating over the other.

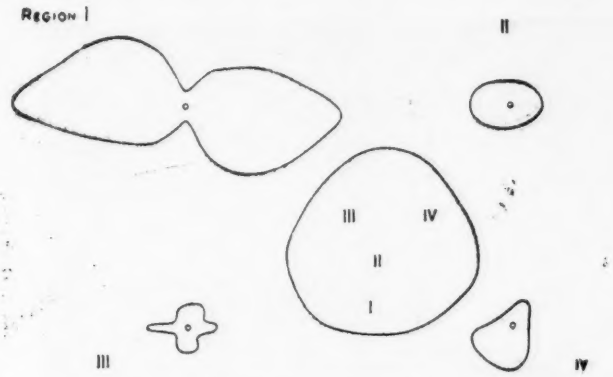


FIG. 1. Directional Variation of Photoconductivity in Diamond N. C. 178.

The second maximum is very weak at I and III, whereas it is quite prominent in the other cases.

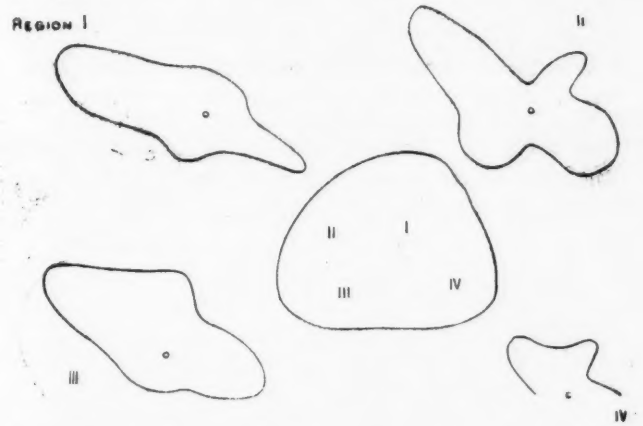
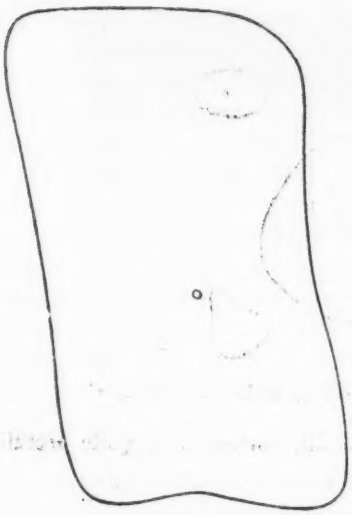


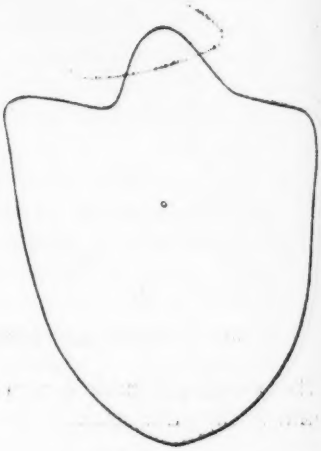
FIG. 2. Directional Variation of Photoconductivity in Diamond N. C. 174.

N. C. 124 and 181.—Both these do not show any striking local variations in conductivity so that in each case, only the central region was studied. The curves obtained are reproduced in Figs. 3 and 4, the different directions being the same as in the pictures shown in the plate.

N. C. 60.—In this case, as mentioned earlier, only a particular region of it, which was weakly birefringent, could be studied and the curve for that



3



4

FIG. 3. Directional Variation of Photoconductivity in N. C. 124.
FIG. 4. Directional Variation of Photoconductivity in N. C. 181.

region is given in Fig. 5. The horizontal axis is supposed to coincide with the direction of the shorter edges. The direction of the maximum conduction is exactly the same as that of the streaks.

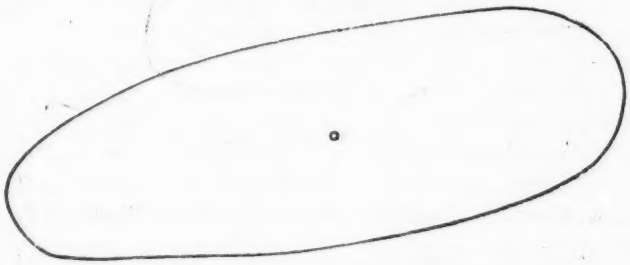


FIG. 5. Directional Variation of Photoconductivity in N. C. 60.

On a comparative study of the birefringence photographs and the polar diagrams reproduced herewith, one cannot but be struck by the close resemblance between the directions of the streaks in the former and the directions of the maxima in the latter. It seems evident therefore that the directional variation of the conductivity is in some way related to the presence of these

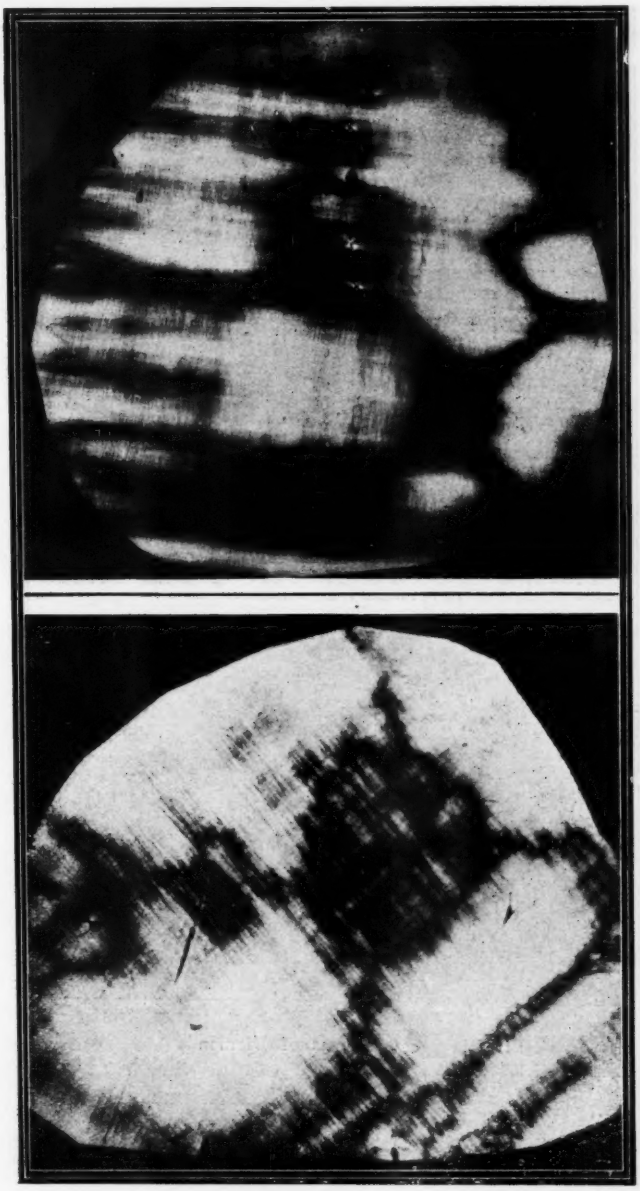


FIG. 1
N.C. 178

FIG. 2
N.C. 174

Birefringence Patterns of Diamonds

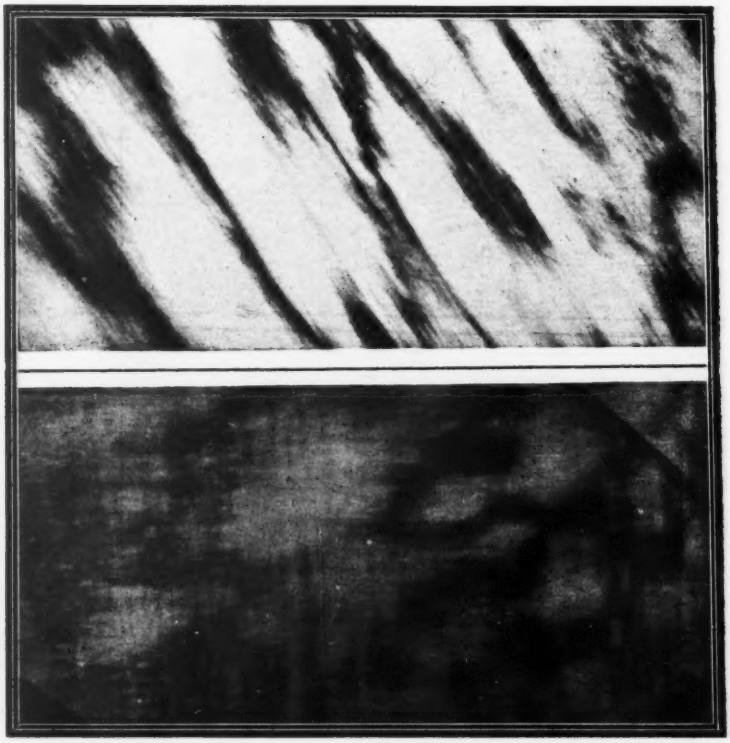


FIG 3
N.C. 124

FIG. 4
N.C. 181

Birefringence Patterns of Diamonds

birefringence streaks and hence naturally with the laminæ associated with them. The gradual extinction of the horizontal maximum as one passes from curve I to curve IV in Fig. 1 and the presence of two maxima at right angles to each other in Fig. 2 for N. C. 174 support this view-point. This effect is probably due to a check by the laminations of the motion of electric charges across them, thereby producing a maximum of conductivity along their direction and a minimum at right angles to it. According to G. N. Ramachandran, the laminæ are clear regions of discontinuity, so that it is quite likely that such a retardation of the electrons occurs.

Another general point of observation is that the conductivity is greater in the regions of a diamond where the birefringence is weak than elsewhere. As proof for this, both the diamonds N. C. 60 and 178 show particularly large local variations in conductivity, which, in general, follow the above observation. Special attention can be drawn to region I of N. C. 178 and that part of N. C. 60 where it shows weak, streaky birefringence. In both these regions, the conductivity is relatively greater. Similarly N. C. 124 and 181 which are almost uniformly birefringent, do not show this effect to any remarkable degree.

The phenomena described in the paper were observed in N. C. 178 and the results were reported in the thesis for the M.Sc. degree submitted by the author to the Madras University in August 1946. The work was repeated with the other diamonds in the summer of 1947 and the paper in its final form was ready for publication in December 1947. Finally, the author wishes to express his sense of gratitude to Sir C. V. Raman at whose suggestion the investigation was undertaken.

Summary

Five cleavage plates of diamond of varying thicknesses were studied as regards their photo-conducting behaviour. All of them show variations of this conductivity with direction. It has been observed that the conductivity is maximum in the direction of some laminæ inside them which make their presence felt by producing sets of streaks in the birefringence pattern photographs. A probable reason for this effect has also been suggested. It is further observed that in general, wherever the birefringence is weak, the relative value of the photoconductivity is greater than in other regions.

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ERRATUM

Vol. XXVI, No. 6, *December* 1947, *Sec. A*

Page 387.—4th line of section (4) *for* 93 *read* 165.

Page 404.—In the description of Fig. *for* cm^{1} *read* cm^{-1}.

Page 409.—Last line *for* les *read* less.

Page 425.—In Table I under 3rd order spectrum entry No. 1, *for* $2\nu_6$ *read* $3\nu_6$.

Page 444.—1st line, *for* exhibts *read* exhibits.

Page 465.—Description of Fig. 4, *for* ptoaz *read* topaz.

Page 472.—4th line, *for* N.C. 175 *read* N.C. 177.

Page 477.—12th line, *for* layers *read* equivalent layers.

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Bergmann and Stather . . . . . Z. Physiol. Chem., 1926, 152, 189.

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